ASSESSMENT OF IN VITRO AND ANTI TUMOUR ACTIVITIES OF AQUEOUS AND METHANOLIC EXTRACTS OF *ABUTILON INDICUM LINN.*

A Dissertation submitted to

The Tamilnadu Dr.M.G.R.Medical University

Chennai-600 032

In Partial fulfillment of the requirements for the award of Degree of

Master of Pharmacy

In

PHARMACOLOGY

By

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Kumarapalayam

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EVALUVATION CERTIFICATE

This is to certify that the dissertation work entitle **"Assessment of In Vitro And Anti Tumour Activities Of Aqueous And Methanolic Extracts Of *Abutilon Indicum Linn".*** submitted by the student bearing **Register no: 261525213** to "**The Tamil Nadu Dr M.G.R Medical University – Chennai**", in practical fulfillment for the award of **Degree of Master of Pharmacy in Pharmacology** is the bonafide work carried out under the guidance and direct supervision of **Dr. R.Shanmuga Sundaram** Head of the Department of Pharmacology and was evaluated by us during the examination held on ……..

Internal Examiner **External Examiner**

CERTIFICATE

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Dr. R. Shanmugasundaram, M.Pharm., Ph. D **Dr.R.Sambath Kumar, M.Pharm., Ph.D**

Guide & HOD **Principal**

CERTIFICATE

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DECLARATION

I do hereby declare that the dissertation work entitled **"Assessment of In Vitro And Anti Tumour Activities Of Aqueous And Methanolic Extracts Of *Abutilon Indicum Linn*".** submitted by the student bearing **Register no: 261525213** to "**The Tamil Nadu Dr M.G.R Medical University – Chennai**", in practical fulfillment for the award of **Degree of Master of Pharmacy in Pharmacology** was done under the guidance of **Dr. R.Shanmuga Sundaram., M.Pharm., Ph.D** at the Department of pharmacology, J.K.K. Nattraja College of Pharmacy, Kumarapalayam, during the academic year 2016- 2017.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

Place : Kumarapalayam **SUSAN ALBY**

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SUSAN ALBY

CONTENTS

1. INTRODUCTION

1.1.MEDICINAL PLANTS

India has a rich Culture of Medicinal herbs and spices, Which includes about more than 2000 species and has a vast geographical area With high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only few have been studied chemically and Pharmacologically for their potential medicinal value [(1)].

Herbal molecules are safe and could overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell.

1.2. ROLE OF HERBAL MEDICINES IN HEALTH CARE SYSTEM

Medicinal herbs play an important role in primary health care system among rural population since synthetic anti-cancer remedies are beyond the reach of common man because of the cost factor. The herbal medicines have a vital role in the prevention and treatment of cancer. Which execute their therapeutic effect by inhibiting cancer activating ezymes and hormones , stimulating DNA repair mechanism. [(2)].

People living in rural areas from their personal experience know that these traditional remedies are valuable source of natura l products to maintain human health, but they may not understand the science behind these medicines, but know that some medicinal plants are highly effective only when used at therapeutic doses.

A survey of current pharmaceutical use revealed that of the total prescription drugs dispensed 25% are plant derived. Plant compounds are highly varied in structure; many are aromatic substance which are phenols or their oxygen –substituted derivatives. However, there is an increased attention on extracts and biologically active compounds isolated from plant species used in herbal medicine, due to its side effects and the resistance that pathogenic micro-organism build against the antibiotics.

Plant constituents may be isolated and used directly as therapeutic agents or as starting materials for drug synthesis or they may serve as models for pharmacologically active compounds in drug synthesis. The general research methods includes proper selection of medicinal plant preparation of crude extract ,biological screening, detailed chemo pharmacological investigations, toxicological and clinical

studies, standardization and use of active moiety as the lead molecule for drug design [3]

1.3. ANCIENT INDIAN SYSTEM OF MEDICINE

Ayurveda is an ancient Indian system of medicine that makes use of herbs existing in the nature and formulates herbal remedies using the inherent power present in these herbs. [4]

Ayurveda uses the inherent power of natural herbs to bring about wonderful results on human body. The herbs are natural and 100% safe. Cancer in ayurveda helps stop the abnormal growth of cells. In later stages of the cancer help relieve pain and anxiety. It helps relive patient from the unearthly pain and suffering.

Herbal medicines have a vital role in the prevention and treatment of cancer. A great deal of Pharmaceutical research done in technologically advanced countries like USA, Germany, France, Japan and China has considerably improved quality of the herbal medicines used in the treatment of cancer.[5]

With advanced knowledge of molecular science and refinement in isolation and structure elucidation techniques, we are in a much better position to identify various anticancer herbs is executed by inhibiting cancer activing enzymes, stimulating DNA repair mechanism, promoting production of protective enzymes, inducing antioxidant action by enhancing activity of the immune cells, some protect the body from cancer by enhancing detoxification functions of the body. Certain biological response modifiers derived from herbs are to known to inhibit growth of cancer by modulating the activity of specific hormones and enzymes. Some herbs reduce toxic side effects of chemotherapy and radiotherapy .

Scientist all over the world are concentrating on the herbal medicines to boost immune cells of the body against cancer. By understanding the complex synergistic interaction of various constituents of anti cancer herbs, the herbal formulations can be designed to attack the cancerous cells without harming normal cells of the body.[6]

1.4. TUMOR

It is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body Not all tumors are cancerous; benign tumors do not spread to other parts of the body Possible signs and symptoms include a lump, abnormal bleeding, prolonged cough, unexplained weight loss and a change in bowel movements. While these symptoms may indicate cancer, they may have other causes Over 100 types of cancers affect humans.

1.5 COLLECTION OF RELATED DISEASE:

Cancer is the name given to a collection of related diseases. In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissues. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and divide to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place.

When cancer develops, however, this orderly process breaks down. As cells become more and more abnormal, old or damaged cells survive when they should die, and new cells form when they are not needed. These extra cells can divide without stopping and may form growths called tumors. [7]

Many cancers form solid tumors, which are masses of tissue. Cancers of the blood, such as leukemias, generally do not form solid tumors. Cancerous tumors are malignant, which means they can spread into, or invade, nearby tissues. In addition, as these tumors grow, some cancer cells can break off and travel to distant places in the body through the blood or the lymph system and form new tumors far from the original tumor.

Unlike malignant , benign tumors do not spread into, or invade, nearby tissues. This can sometimes be quite large, however. When removed, they usually don't grow back, whereas malignant tumors sometimes do. Unlike most benign tumors elsewhere in the body, benign brain tumors can be life threatening.

1.5.DIFFERENCE BETWEEN CANCER CELLS AND NORMAL CELLS :

Cancer cells differ from normal cells in many ways that allow them to grow out of control and become invasive. One important difference is that cancer cells are less specialized than normal cells. That is, whereas normal cells mature into very distinct cell types with specific functions, cancer cells do not. This is one reason that, unlike normal cells, cancer cells continue to divide without stopping. (8)

In addition, cancer cells are able to ignore signals that normally tell cells to stop dividing or that begin a process known as programmed cell death, or apoptosis, which the body uses to get rid of un needed cells.

Cancer cells may be able to influence the normal cells, molecules, and blood vessels that surround and feed a tumor an area known as the micro environment. For instance, cancer cells can induce nearby normal cells to form blood vessels that supply tumors with oxygen and nutrients, which they need to grow. These blood vessels also remove waste products from tumors.

Cancer cells are also often able to evade the immune system, a network of organs, tissues, and specialized cells that protects the body from infections and other conditions. Although the immune system normally removes damaged or abnormal cells from the body, some cancer cells are able to "hide" from the immune system.(9)

Tumors can also use the immune system to stay alive and grow. For example, with the help of certain immune system cells that normally prevent a run away immune response cancer cells can actually keep the immune system from killing cancer cells.

1.6. HOW CANCER ARISES

Cancer is a genetic disease that , it is caused by changes to genes that control the way our cells function, especially how they grow and divide.

Genetic changes that cause cancer can be inherited from our parents. They can also arise during a person's lifetime as a result of errors that occur as cells divide or

because of damage to DNA caused by certain environmental exposures. Cancer-causing environmental exposures include substances, such as the chemicals in tobacco smoke, and radiation, such as ultraviolet rays from the sun. Each person's cancer has a unique combination of genetic changes. As the cancer continues to grow, additional changes will occur. Even within the same tumor, different cells may have different genetic changes.

In general, cancer cells have more genetic changes, such as mutations in DNA, than normal cells. Some of these changes may have nothing to do with the cancer; they may be the result of the cancer, rather than its cause.

1.7. DRIVERS OF CANCER

The genetic changes that contribute to cancer tend to affect three main types of genes Proto-oncogenesis tumour suressor genes, and DNA repair genes. These changes are sometimes called "drivers" of cancer.

Proto-oncogenes are involved in normal cell growth and division. However, when these genes are altered in certain ways or are more active than normal, they may become cancer-causing genes (or oncogenes), allowing cells to grow and survive when they should not.[10] Tumor suppressor genes are also involved in controlling cell growth and division. Cells with certain alterations in tumor suppressor genes may divide in an uncontrolled manner.

DNA repair genes are involved in fixing damaged DNA. Cells with mutations in these genes tend to develop additional mutations in other genes. Together, these mutations may cause the cells to become cancerous.

As scientists have learned more about the molecular changes that lead to cancer, they have found that certain mutations commonly occur in many types of cancer. Because of this,

cancers are sometimes characterized by the types of genetic alterations that are believed to be driving them, not just by where they develop in the body and how the cancer cells look under the microscope.[11]

1.7.WHEN CANCER SPREADS

A cancer that has spread from the place where it first started to another place in the body is called metastatic cancer. The process by which cancer cells spread to other parts of the body is called metastasis.[12]

Metastatic cancer has the same name and the same type of cancer cells as the original, or primary, cancer. For example, breast cancer that spreads to and forms a metastatic tumor in the lung is metastatic breast cancer, not lung cancer.

Under a microscope, metastatic cancer cells generally look the same as cells of the original cancer. Moreover, metastatic cancer cells and cells of the original cancer usually have some molecular features in common, such as the presence of specific chromosome changes.

Metastasis is the spread of cancer to other locations in the body. The dispersed tumors are called metastatic tumors, while the original is called the primary tumor. Almost all cancers can metastasize. Most cancer deaths are due to cancer that has metastasized.. It is common in the late stages of cancer and it can occur via the blood or the lymphatic system or both. The typical steps in metastasis are local invasion, intravasation into the blood or lymph, circulation through the body, extravasation into the new tissue, proliferation and angiogenesis. Different types of cancers tend to metastasize to particular organs, but overall the most common places for metastases to occur are the lungs, liver, brain and the bones.

In Metastasis, Cancer cells break away from where they first formed (primary cancer), travel through the blood lymph system, and form new tumors (metastic tumors) in other parts of the body.

Treatment may help prolong the lives of some people with metastatic cancer. In general, though, the primary goal of treatments for metastatic cancer is to control the growth of the cancer or to relieve symptoms caused by it. It can cause severe damage to how the body functions, and most people who die of cancer die of metastatic disease.

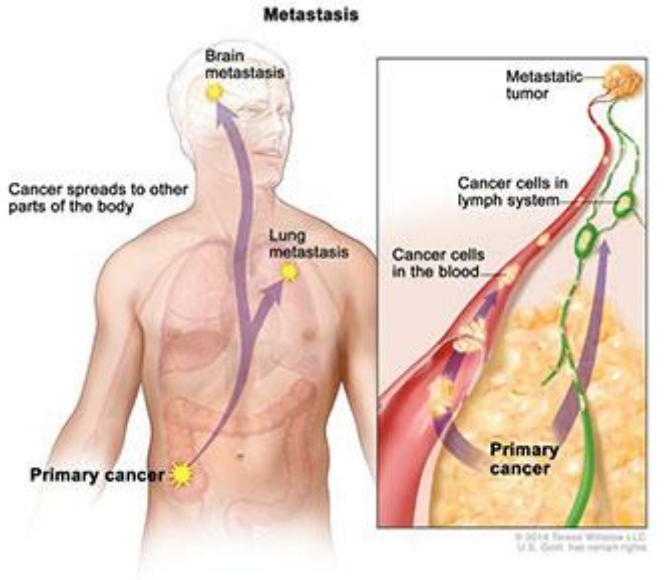

Fig.no.1

1.8.TISSUE CHANGES THAT ARE NOT A CANCER

Not every change in the body's tissues is cancer. Some tissue changes may develop into cancer if they are not treated, however. Here are some examples of tissue changes that are not cancer but, in some cases, are monitored:

Hyperplasia occurs when cells within a tissue divide faster than normal and extra cells build up, or proliferate. However, the cells and the way the tissue is organized look normal under a microscope. Hyperplasia can be caused by several factors or conditions, including chronic irritation.(13)

Dysplasia is a more serious condition than hyperplasia. In dysplasia, there is also a build up of extra cells. But the cells look abnormal and there are changes in how the tissue is organized. In general, the more abnormal the cells and tissue look, the greater the chance that cancer will form.(14)

Some types of dysplasia may need to be monitored or treated. An example of dysplasia is an abnormal mole (called a dysplastic nevus) that forms on the skin. A dysplastic nerves can turn into melanoma, although most do not.

An even more serious condition is carcinoma in situ. Although it is sometimes called cancer, carcinoma in site is not cancer because the abnormal cells do not spread beyond the original tissue. That is, they do not invade nearby tissue the way that cancer cells do. But, because some carcinomas in situ may become cancer, they are usually treated. (14)

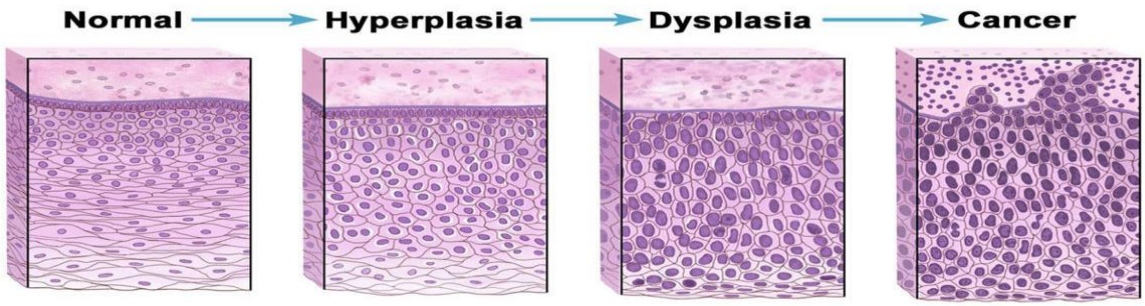

Fig.No. 2.

Normal cells may become cancer cells form in tissues of the body, the cells go through abnormal changes called hyperplasia and dysplasia. In hyperplasia, there is an increase in the number of cells in an organ or tissue that appear normal under a microscope. In dysplasia, the cells look abnormal under a microscope but are not cancer. Hyperplasia and dysplasia may or may not become cancer.

1.9.TYPES OF CANCER

There are more than 100 types of cancer. Types of cancer are usually named for the organs or tissues where the cancers form. For example, lung cancer starts in cells of the lung, and brain cancer starts in cells of the brain. Cancers also may be described by the type of cell that formed them, such as an epithelial cell or a squamous cell. (14)

Here are some categories of cancers that begin in specific types of cells.

CARCINOMA

Carcinomas are the most common type of cancer. They are formed by epithelial cells, which are the cells that cover the inside and outside surfaces of the

body. There are many types of epithelial cells, which often have a column-like shape when viewed under a microscope.

Carcinomas that begin in different epithelial cell types have specific names:

ADENOCARCINOMA

It is a cancer that forms in epithelial cells that produce fluids or mucus. Tissues with this type of epithelial cell are sometimes called glandular tissues. Most cancers of the breast, colon, and prostate are adenocarcinomas.

BASAL CELL CARCINOMA

It is a cancer that begins in the lower or basal (base) layer of the epidermis, which is a person's outer layer of skin.

SQUAMOUS CELL CARCINOMA

It is a cancer that forms in squamous cells, which are epithelial cells that lie just beneath the outer surface of the skin. Squamous cells also line many other organs, including the stomach, intestines, lungs, bladder, and kidneys. Squamous cells look flat, like fish scales, when viewed under a microscope. Squamous cell carcinomas are sometimes called epidermoid carcinomas.(15).

TRANSITIONAL CELL CARCINOMA

It is a cancer that forms in a type of epithelial tissue called transitional epithelium, or urothelium. This tissue, which is made up of many layers of epithelial cells that can get bigger and smaller, is found in the linings of the bladder, ureters, and part of the kidneys (renal pelvis), and a few other organs. Some cancers of the bladder, ureters, and kidneys are transitional cell carcinomas.

SARCOMA

Sarcomas are cancers that form in bone and soft tissues, including muscle, fat, blood vessels, lymph vessels and fibrous tissue (such as tendons and ligaments). Osteosarcoma is the most common cancer of bone.

LEUKIMIA

Cancers that begin in the blood-forming tissue of the bone marrow are called leukemias. These cancers do not form solid tumors. Instead, large numbers of abnormal white blood cells (leukemia cells and leukemic blast cells) build up in the blood and bone marrow, crowding out normal blood cells. The low level of normal

blood cells can make it harder for the body to get oxygen to its tissues, control bleeding, or fight infections.[16]

LYMPHOMA

Lymphoma is cancer that begins in lymphocytes (T cells or B cells). These are disease-fighting white blood cells that are part of the immune system. In lymphoma, abnormal lymphocytes build up in lymph nodes and lymph vessels, as well as in other organs of the body. [14]

OTHER TYPES OF TUMOURS

GERM CELL TUMOUR

Germ cell tumors are a type of tumor that begins in the cells that give rise to sperm or eggs. These tumors can occur almost anywhere in the body and can be either benign or malignant.[17]

NEUROENDOCRINE TUMOURS

Neuroendocrine tumors form from cells that release hormones into the blood in response to a signal from the nervous system. These tumors, which may make higher-than-normal amounts of hormones, can cause many different symptoms. Neuroendocrine tumors may be benign or malignant

CARCINOID TUMOURS

Carcinoid tumors are a type of neuroendocrine tumor. They are slow-growing tumors that are usually found in the gastrointestinal system (most often in the rectum and small intestine). Carcinoid tumors may spread to the liver or other sites in the body, and they may secrete substances such as serotonin or prostaglandins, causing carcinoid syndrome. [18]

COMMON TYPES OF CANCER

Cancer is a common condition and is a serious health problem, both in the UK and across the world. It is estimated that 7.6 million people in the world died of cancer in 2007. In the UK, cancer is responsible for 126,000 deaths per year. One in four people die from cancer.

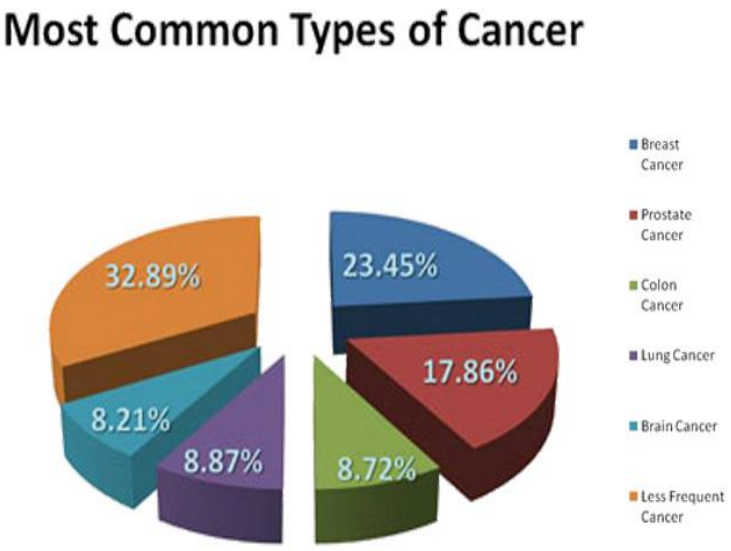

Fig. No: 3

There are hundreds of different types of cancer. The most common cancers are:

1.Breast cancer,
2. Prostate cancer,
3. Lung cancer,
4. Cancer of colon or rectum,
5. Blood cancer,
6. Bladder cancer,
7. Liver Cancer,
8. Ovarian cancer

1.13. RISK FACTORS OF CANCER

- It is usually not possible to know exactly why one person develops cancer and another doesn't. But research has shown that certain risk factors may increase a person's chances of developing cancer.

- Cancer risk factors include exposure to chemicals or other substances, as well as certain behaviors. They also include things people cannot control, like age and family history.

- A family history of certain cancers can be a sign of a possible inherited cancer syndrome. Most cancer risk (and protective) factors are initially identified in epidemiology studies.

- Although some of these risk factors can be avoided, others-such as growing older-cannot. Limiting your exposure to avoidable risk factors may lower your risk of developing certain cancers
- Age
- Alcohol
- Cancer-Causing Substances
- Chronic Inflammation
- Diet
- Hormones
- Immunosuppression
- Infectious Agents
- Obesity
- Radiation
- Sunlight
- Tobacco

1.15. COLORECTAL CANCER (CRC)

It also known as bowel cancer and colon cancer, is the development of cancer from the colon or rectum (parts of the large intestine). A cancer is the abnormal growth of cells that have the ability to invade or spread to other parts of the body. Signs and symptoms may include blood in the stool, a change in bowel movements, weight loss, and feeling tired all the time[19]

Most colorectal cancers are due to old age and lifestyle factors with only a small number of cases due to underlying genetic disorders. Some risk factors include diet, obesity, smoking, and lack of physical activity.

Dietary factors that increase the risk include red and processed meat as well as alcohol, Another risk factor is inflammatory bowel disease which includes Crohn's disease and ulcerative colitis. Some of the inherited genetic disorders that can cause

colorectal cancer include familial adenomatous polyposis and hereditary non-polyposis colon cancer, however, these represent less than 5% of cases It typically starts as a benign tumor, often in the form of a polyp, which over time becomes cancerous[20].

COLON CANCER

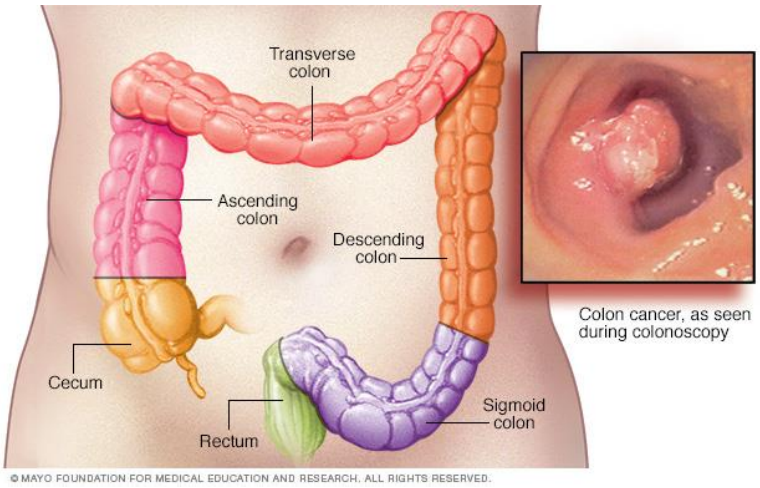

Fig. No: 4

1.16. SIGNS AND SYMPTOMS

The classic warning signs include:

- worsening constipation,
- blood in the stool,
- decrease in stool caliber (thickness),
- loss of appetite,
- loss of weight,
- Nausea or vomiting in someone over 50 years old.

1.17. CAUSES

Greater than 75–95% of colorectal cancer occurs in people with little or no genetic risk.Risk factors include older age, male gender high intake of fat, alcohol, red meat, processed meats, obesity, smoking, and a lack of physical exercise.Approximately 10% of cases are linked to insufficient activity The risk for alcohol appears to increase

at greater than one drink per day Drinking 5 glasses of water a day is linked to a decrease in the risk of colorectal cancer and adenomatous polyps.[21]

1.18. INFLAMMATORY BOWEL DISEASE

People with inflammatory bowel disease (ulcerative colitis and Crohn's disease) are at increased risk of colon cancer. The risk increases the longer a person has the disease, and the worse the severity of inflammation. In these high risk groups, both prevention with aspirin and regular colonoscopies are recommended. People with inflammatory bowel disease account for less than 2% of colon cancer cases yearly. In those with Crohn's disease 2% get colorectal cancer after 10 years, 8% after 20 years, and 18% after 30 years[22]. In those with ulcerative colitis approximately 16% develop either a cancer precursor or cancer of the colon over 30 years.

1.19. GENETICS

Those with a family history in two or more first-degree relatives (such as a parent or sibling) have a two to three fold greater risk of disease and this group accounts for about 20% of all cases. A number of genetic syndromes are also associated with higher rates of colorectal cancer.

1.21. LUNG CANCER

It also known as lung carcinoma is a malignant lung tumour characterized by uncontrolled cell growth in tissues of the lung. If left untreated, this growth can spread beyond the lung by the process of metastasis into nearby tissue or other parts of the body. Most cancers that start in the lung, known as primary lung cancers, are carcinomas. The two main types are small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC). The most common symptoms are coughing (including coughing up blood, weight loss, shortness of breath, and chest pains.[23]

The vast majority (85%) of cases of lung cancer are due to long-term tobacco smoking, About 10–15% of cases occur in people who have never smoked, These cases are often caused by a combination of genetic factors and exposure to radon gas, asbestos, second-hand smoke, or other forms of air pollution, Lung cancer may be

seen on chest radiographs and computed tomography (CT) scan. The diagnosis is confirmed by biopsy which is usually performed by bronchoscopy or CT-guidance.

Avoidance of risk factors, including smoking and air pollution, is the primary method of prevention. Treatment and long-term outcomes depend on the type of cancer, the stage (degree of spread), and the person's overall health. Most cases are not curable Common treatments include surgery, chemotherapy, and radiotherapy.NSCLC is sometimes treated with surgery, whereas SCLC usually responds better to chemotherapy and radiotherapy..

Worldwide in 2012, lung cancer occurred in 1.8 million people and resulted in 1.6 million deaths. This makes it the most common cause of cancer-related death in men and second most common in women after breast cancer. The most common age at diagnosis is 70 years. Overall, 17.4% of people in the United States diagnosed with lung cancer survive five years after the diagnosis, while outcomes on average are worse in the developing world[24]

LUNG CANCER

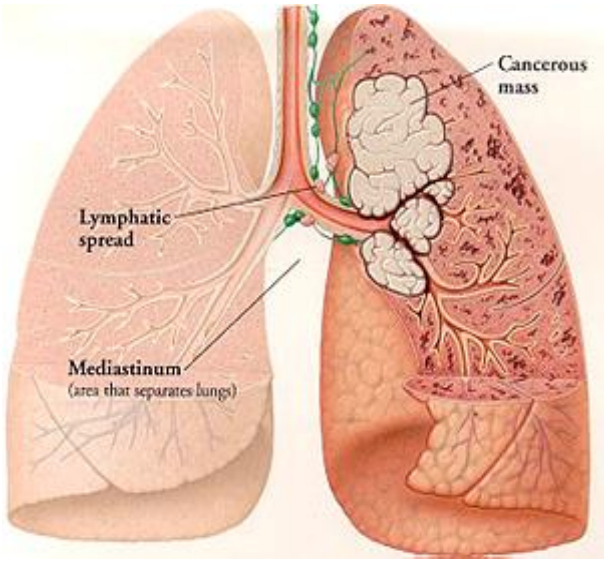

Fig. No: 5

1.23. SIGNS AND SYMPTOMS

Signs and symptoms which may suggest lung cancer include:

- Respiratory symptoms: coughing, coughing up blood, wheezing, or shortness of breath
- Systemic symptoms: weight loss, weakness, fever, or clubbing of the fingernails
- Symptoms due to the cancer mass pressing on adjacent structures: chest pain, bone pain, superior vena cava obstruction, or difficulty swallowing.

1.24. RISK FACTOR

- Cancer develops following genetic damage to DNA and epigenetic changes. These changes affect the normal functions of the cell.
- Smoking, particularly of cigarettes, is by far the main contributor to lung cancer. Cigarette smoke contains at least 73 known carcinogens, including benzo[*a*]pyrene, NNK.
- Passive smoking the inhalation of smoke from another's smoking is a cause of lung cancer in nonsmokers. A passive smoker can be defined as someone living or working with a smoker.
- Outdoor air pollutants, especially chemicals released from the burning of fossil fuels, increase the risk of lung cancer. Fine particulates ($PM_{2.5}$) and sulfate aerosols, which may be released in traffic exhaust fumes.

1.25. LIVER CANCER

It also known as hepatic cancer and primary hepatic cancer, is cancer that starts in the liver, Cancer which has spread from elsewhere to the liver, known as liver metastasis is more common than that which starts in the liver. Symptoms of liver cancer may include a lump or pain in the right side below the rib cage, swelling of the abdomen yellowish skin, easy bruising, weight loss, and weakness[(25).] The leading cause of liver cancer is cirrhosis due to either hepatitis B hepatitis C or alcohol Other causes include aflatoxin non-alcoholic fatty liver disease, and liver flukes.

The most common types are hepatocellular carcinoma (HCC), which makes up 80% of cases, and cholangiocarcinoma. Less common types include mucinous cystic neoplasm and intraductal papillary biliary neoplasm. The diagnosis may be supported by blood tests and medical imaging with confirmation by tissue biopsy.

Preventive efforts include immunization against hepatitisB and treating those infected with hepatitis B or C. Screening is recommended in those with chronic liver disease .

LIVER CANCER

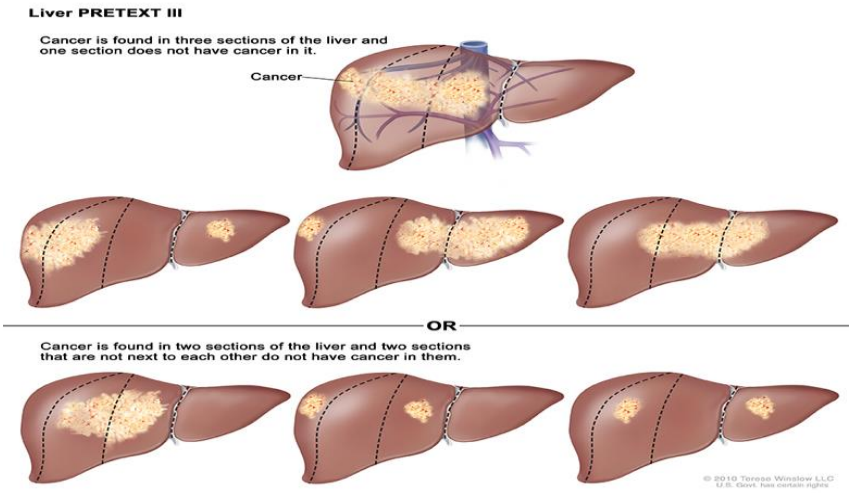

Fig. No: 6

In certain cases ablation therapy, embolization therapy, or liver transplantation, may be used. Small lumps in the liver may be simply closely followed. The word "hepatic" is from the Greek *hêpar*, meaning "liver".

1.26. SIGNS AND SYMPTOMS

Signs and symptoms depend on what type of cancer is present :

The Cholangiocarcinoma is associated with

- Sweating,
- Jaundice,
- Abdominal pain,

- Weight loss and liver enlargement.

Hepatocellular carcinoma is associated with

- Abdominal mass,
- Abdominal pain,
- Emesis, anemia,
- Back pain,
- Jaundice, itching,
- Weight loss and fever.

1.27. CAUSES

VIRUSE INFECTION

Viral infection with either hepatitis C virus (HCV) or Hepatitis B virus (HBV) is the chief cause of liver cancer in the world today, accounting for 80% of hepato cellular carcinoma (HCC) The viruses cause HCC because massive inflammation, fibrosis and eventual cirrhosis occurs within the liver. HCC usually arises after cirrhosis, with an annual incidence of 1.7% in cirrhotic HCV-infected individuals[(26)]Around 5-10% of individuals that become infected with HBV become chronic carriers, and around 30% of these acquire chronic liver disease, which can lead to HCC. HBV infection is also linked to cholangiocarcinoma. The role of viruses other than HCV or HBV in liver cancer is much less clear, although there is some evidence that co-infection of HBV and hepatitis D virus may increase the risk of HCC.

Many genetic and epigenetic changes are formed in liver cells during HCV and HBV infection, which is a major factor in the production of the liver tumors. The viruses induce malignant changes in cells by altering gene methylation, affecting gene expression and promoting or repressing cellular signal transduction pathways. By doing this the viruses can prevent cells from undergoing a programmed form of cell death (apoptosis) and promote viral replication and persistence.

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Aflatoxin exposure can lead to the development of HCC. The aflatoxins are a group of chemicals produced by the fungi *Aspergillus flavus* (the name comes from *A. flavus* toxin) and *A. parasiticus*. Food contamination by the fungi leads to ingestion of the chemicals, which are very toxic to the liver. Common foodstuffs contaminated with the toxins are cereals, peanuts and other vegetables. Contamination of food is common in Africa, South-East Asia and China. Concurrent HBV infection and aflatoxin exposure increases the risk of liver cancer to over three times that seen in HBV infected individuals without aflatoxin exposure. The mechanism by which aflatoxins cause cancer is through genetic mutation of a gene required for the prevention of cancer.

- High grade dysplastic nodules are precancerous lesions of the liver. Within 2 years, there is a risk of cancer arising from these nodules of 30-40%.
- Obesity has emerged as an important risk factor as it can lead to steatohepatitis.
- Diabetes increases the risk of HCC.
- Smoking increases the risk of HCC compared to non-smokers and previous smokers.
- There is around 5-10% lifetime risk of cholangiocarcinoma in people with primary sclerosing cholangitis.

- Liver fluke infection increases the risk of cholangiocarcinoma, and is the reason Thailand has particularly high rates of this cancer.

1.28. CAUSES IN CHILDREN

Increased risk of liver cancer in children can be caused by Beckwith wiedemann Syndrome. (associated with hepatoblastoma,[28] familial adenomatous polyposis (associated with hepatoblastoma), low birth weight (associated with hepatoblastoma). Progressive familial intrahepatic cholestasis (associated with HCC) and Trisomy (associated with hepatoblastoma)

. (1.29.BREAST CANCER

Cancer that develops from breast tissue. Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, or a red scaly patch of skin. In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, shortness of breath, or yellow skin.

Risk factors for developing breast cancer include being female, obesity, lack of physical exercise, drinking alcohol, hormone replacement therapy during menopause, ionizing radiation, early age at first menstruation, having children late or not at all, older age, and family history. About 5–10% of cases are due to genes inherited from a person's parents, including BRCA1 and BRCA2 among others. Breast cancer most commonly develops in cells from the lining of milk ducts and the lobules that supply the ducts with milk. Cancers developing from the ducts are known as ductal carcinomas, while those developing from lobules are known as lobular carcinomas. In addition, there are more than 18 other sub-types of breast cancer. Some cancers, such as ductal carcinoma in situ, develop from pre-invasive lesions. The diagnosis of breast cancer is confirmed by taking a biopsy of the concerning lump. Once the diagnosis is made, further tests are done to determine if the cancer has spread beyond the breast .

The balance of benefits versus harms of breast cancer screening is controversial. A 2013 Cochrane review stated that it is unclear if mammographic screening does more good or harm. A 2009 review for the US Preventive Services

Task Force found evidence of benefit in those 40 to 70 years of age and the organization recommends screening every two years in women 50 to 74 years old[29].

CHANGES DURING BREAST CANCER

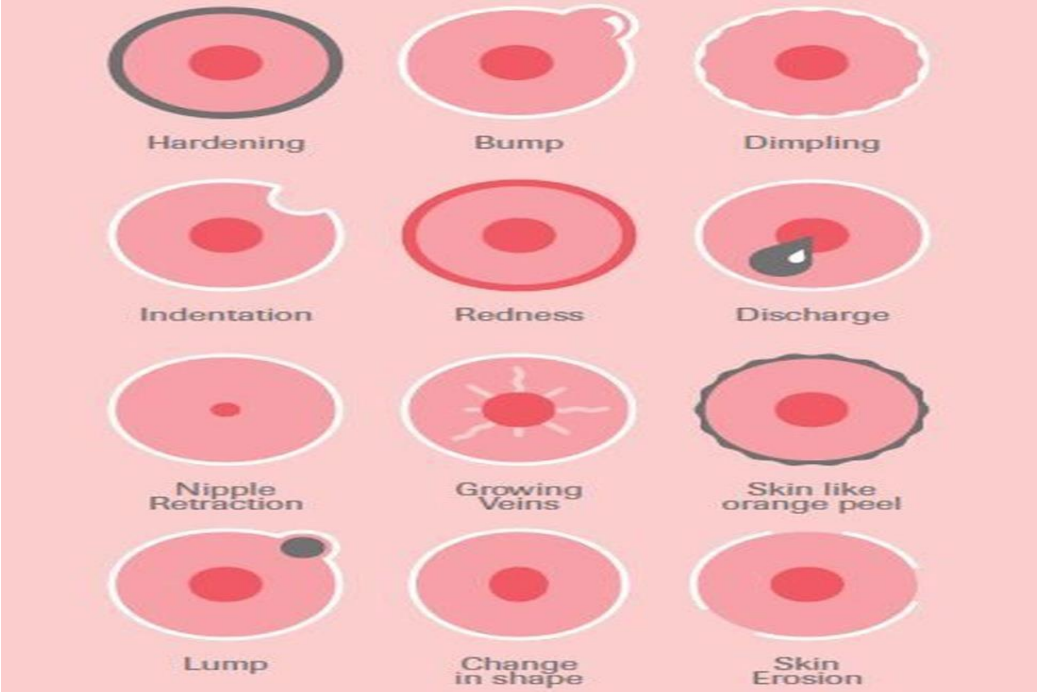

Fig. No: 7

1.30. RISK FACTORS

Risk factors can be divided into two categories:

- ***modifiable* risk** factors (things that people can change themselves, such as consumption of alcoholic beverages), and
- ***fixed* risk** factors (things that cannot be changed, such as age and biological sex).
- The primary risk factors for breast cancer are being female and older age. Other potential risk factors include genetics, lack of childbearing or lack of breastfeeding, higher levels of certain hormones [31].

- Smoking tobacco appears to increase the risk of breast cancer, with the greater the amount smoked and the earlier in life that smoking began, the higher the risk.
- A number of dietary factors have been linked to the risk for breast cancer. Dietary factors which may increase risk include a high fat diet, high alcohol intake, and obesity-related high cholesterol levels.

1.32. PROSTATE CANCER

It is a cancer that occurs in a man's prostate a small walnut shaped that produces the seminal fluid that nourishes and transports sperm. Prostate cancer, also known as carcinoma of the prostate, is the development of cancer in the prostate, a gland in the male reproductive system. Most prostate cancers are slow growing; however, some grow relatively quickly. The cancer cells may spread from the prostate to other parts of the body, particularly the bones and lymph node. It may initially cause no symptoms In later stages it can lead to difficulty urinating, blood in the urine, or pain in the pelvis, back or when urinating. A disease known as benign prostatic hyperplasia may produce similar symptoms. Other late symptoms may include feeling tired due to low levels of red blood cells

Prostate-specific antigen (PSA) testing increases cancer detection but does not decrease mortality.The United States Preventive Services Task Force recommends against screening using the PSA test, due to the risk of over diagnosis and overtreatment, as most cancer diagnosed would remain asymptomatic. (32).

Many cases can be safely followed with active surveillance or watchful waiting. Other treatments may include a combination of surgery, radiation therapy, hormone therapy or chemotherapy.When it only occurs inside the prostate it may be curable–In those in whom the disease has spread to the bones, pain medications, bisphosphonates and targeted therapy, among others, may be useful.

STAGES OF PROSTATE CANCER

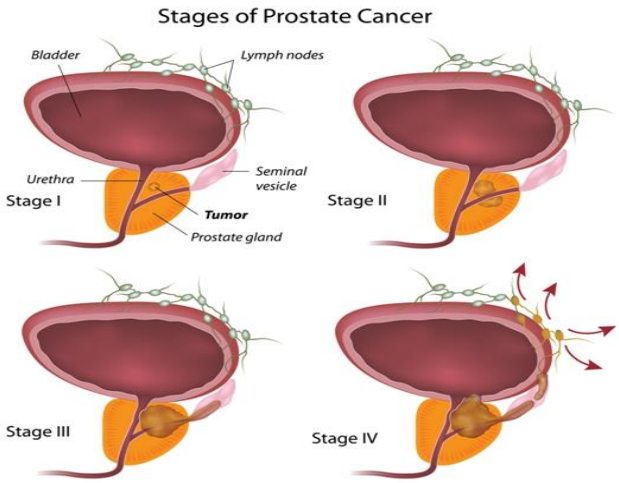

Fig. No: 8

1.33. SIGNS AND SYMPTOMS

- Hematuria (blood in the urine),
- Dysuria (painful urination)
- Nocturia (increased urination at night)
- Leg weakness
- Bone pain
- Painful ejaculation

1.34. RISK FACTORS

- A complete understanding of the causes of prostate cancer remains elusive. The primary risk factors are obesity, age and family history.
- Prostate cancer is very uncommon in men younger than 45, but becomes more common with advancing age. The average age at the time of diagnosis is 70.
- However, many men never know they have prostate cancer. Autopsy studies of Chinese, German, Israeli, Jamaican, Swedish, and Ugandan men who died of other causes have found prostate cancer in 30% of men in their fifties, and in

- 80% of men in their seventies.Men who have first-degree family members with prostate cancer appear to have double the risk of getting the disease compared to men without prostate cancer in the family.

1.34. OVARIAN CANCER

It is a cancer that forms in an ovary. It results in abnormal cells that have the ability to invade or spread to other parts of the body. When this process begins, there may be no or only vague symptoms. Symptoms become more noticeable as the cancer progresse These symptoms may include bloating, pelvic pain, abdominal swelling, and loss of appetite, among other (33). Common areas to which the cancer may spread include the lining of the abdomen ,lining of the bowel and bladder, lymph nodes, lungs ,and liver.(24)

The risk of ovarian cancer increases in women who have ovulated more over their lifetime. This includes those who have never had children, those who begin ovulation at a younger age or reach menopause at an older age. Other risk factors include hormone therapy after menopause, fertility medication, and obesiety. Factors that decrease risk include hormonal birth control, tubal ligation, and breast feeding.

1.35. SIGNS AND SYMPTOMS

Signs and symptoms of ovarian cancer are:

- Bloating,
- Abdomino pelvic pain or discomfort
- Back pain,
- Irregular menstruation or postmenopausal
- Vaginal bleeding,
- Constipation
- Urinary symptoms (including frequent urination and urgent urination).

1.36. RISK FACTORS

- Most of the risk for ovarian cancer is related to the amount of time spent in ovulation. Thus not having children is a risk factor for ovarian cancer, likely because ovulation is suppressed via pregnancy.
- Things that halt ovulation including breast feeding, oral contraceptive use with estrogen/progesterone combination contraceptives, multiple pregnancies, and pregnancy at an early age, all decrease risk of ovarian cancer.
- These conditions decrease the overall time during one's lifetime spent ovulating. A positive family history of ovarian cancer is a risk factor for ovarian cancer.
- People with hereditary nonpolyposis colon cancer (Lynch Syndrome), and those with BRCA-1 and BRCA-2 genetic abnormalities are at increased risk. [25].

1.38. CELL LINES USING IN- VITRO STUDIES

Cancer cell lines have been widely used for research purposes and proved to be a useful tool in the genetic approach, and its characterization shows that they are, in fact, an excellent model for the study of the biological mechanisms involved in cancer. Examples are shown in **Table.1** The use of cancer cell lines allowed an increment of the information about the deregulated genes and signalling pathways in this disease . Furthermore, the use of the cell model was in the origin of the development and testing of anticancer drugs presently used , and in the development of new therapies , but also as an alternative to transplantable animal tumours in chemotherapeutics testing . In fact, the use of the appropriate *in vitro* model in cancer research is crucial for the investigation of genetic, epigenetic and cellular pathways , for the study of proliferation deregulation, apoptosis and cancer progression , to define potential molecular markers and for the screening and characterization of cancer therapeutics . The results of the research in cancer cell lines are usually extrapolated to *in vivo* human tumours and its importance as models for drug testing and translational study have been recognized by many biomedical and pharmaceutical companies. [34]

Table 1

Cancer cell line	Species	Signs	Morphology
mcf-7	Homo sapiens	Breast adenocarcinoma	Epithelial
COLO-205,SW-620	Homo sapiens	Colon adenocarcinoma	Epithelial
A549,NCI-H23	Homo sapiens	Lung carcinoma	Epithelial
HEP-2	Homo sapiens	Hepatocellular carcinoma	Epithelial
OVCAR-5	Homo sapiens	Ovarian Carcinoma	Lymphoblast
DU-145	Homo sapiens	Prostate adenocarcinoma	Epithelial

1.39. CYTOTOXICITY

It is the quality of being toxic to cells. Examples of toxic agents are an immune cells. or some types of venom, e.g. from the puff adder (*Bitis arietans*) or brown recluse spider (*Loxosceles reclusa*).

1.40. CELL PHYSIOLOGY

Treating cells with the cytotoxic compound can result in a variety of cell fates. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis. The cells can stop actively growing and dividing (a decrease in cell viability), or the cells can activate a genetic program of controlled cell death (apoptosis).

1.41. MEASUREMENT

Cytotoxicity assays are widely used by the pharmaceutical industry to screen for cytotoxicity in compound libraries. Researchers can either look for cytotoxic compounds, if they are interested in developing a therapeutic that targets rapidly dividing cancer cells, for instance; or they can screen "hits" from initial high-throughput drug screens for unwanted cytotoxic effects before investing in their development as a pharmaceuticals.

Assessing cell membrane integrity is one of the most common ways to measure cell viability and cytotoxic effects. Compounds that have cytotoxic effects often compromise cell membrane integrity. Vital dyes, such as trypan blue or propidium iodide are normally excluded from the inside of healthy cells; however, if the cell membrane has been compromised, they freely cross the membrane and stain intracellular components. Alternatively, membrane integrity can be assessed by monitoring the passage of substances that are normally sequestered inside cells to the outside.

1.42. CYTOTOXICITY ASSAY

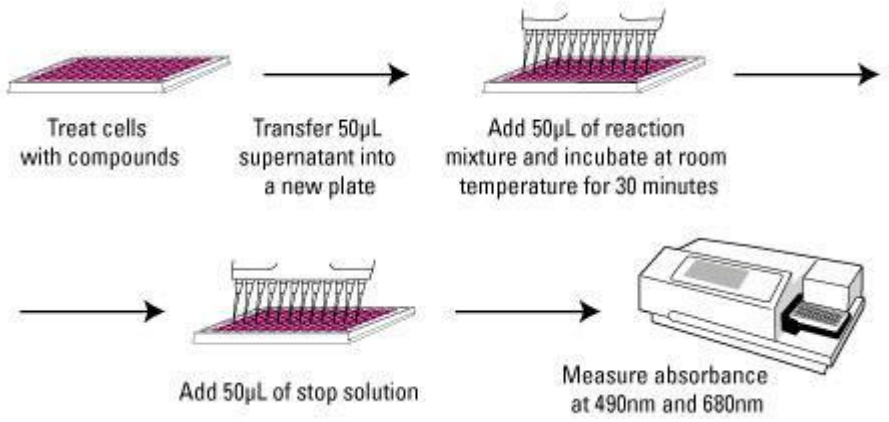

Fig. No: 10

Cell viability and cytotoxicity assays are used for drug screening and cytotoxicity tests of chemicals. indicates various reagents used for cell viability detection. They are based on various cell functions such as enzyme activity, cell membrane permeability, cell adherence, ATP production, co-enzyme production, and nucleotide uptake activity. Many have established methods such as Colony Formation method, Crystal Violet method, Tritium-Labeled Thymidine Uptake method, MTT, and WST methods, which are used for counting the number of live cells. Trypan Blue is a widely used assay for staining dead cells. [35].

In this method, cell viability must be determined by counting the unstained cells with a microscope or other instruments. However, Trypan Blue staining cannot

be used to distinguish between the healthy cells and the cells that are alive but losing cell functions. In the Colony Formation method, the number of cell colonies are counted using a microscope as a cell viability indicator. In the Tritium-Labeled Thymidine Uptake method, [3H]-thymidine is involved in the cell nucleus due to the cell growth, and the amount of the tritium in the nucleus is then measured using a scintillation counter. Though the Tritiumlabeled thymidine uptake assay is sensitive to determine the influence on the DNA polymerization activity, it requires radioisotope which causes various concerns.

The 51Cr method is highly sensitive, and is commonly used to determine low levels of cytotoxicity. However, the use of 51Cr also causes problems in handling, storage, and disposal of the material. Cellular enzymes such as lactate dehydrogenase, adenylate kinase,

and glucose-6-phosphate dehydrogennase are also used as cell death markers, and there are severalproducts available on the market. However, adenylate kinase and glucose-6-phosphate are not stable and only lactate dehydrogenase does not lose its activity during cell death assays. therefore, cell death assays based on lactate dehydrogenase (LDH) activity are more reliable than other enzyme-based cell death assays.

Enzyme-based methods using MTT and WST rely on a reductive coloring reagent and dehydrogenase in a viable cell to determine cell viability with a colorimetric method. This method s far superior to the previously mentioned methods because it is easy-to-use, safe, has a high reproducibility, and is widely used in both cell viability and cytotoxicity tests. Therefore, this method is suitable for those who are just beginning such experiments. Among the enzyme-based assays, the MTT assay is the best known method for determining mitochondrial dehydrogenase activities in the living cells. In the method, MTT is reduced to a purple formazan by NADH. However, MTT formazan is insoluble in water, and it forms purple needle shaped crystals in the cells. Therefore prior to measuring the absorbance, an organic solvent is required to solubilize the crystals. Additionally, the cytotoxicity of MTT formazan makes it difficult to remove cell culture media from the plate wells due to floating cells with MTT formazan needles, giving significant well-to-well error.

Produce water-soluble formazans and are suitable for cell proliferation and cytotoxicity assays. WSTs receives two electrons from viable cells to generate a yellow, orange, or purple formazan dye. WST-8, a highly stable WST, is utilized in Cell Counting Kit-8 (CCK-8). The electron mediator used in this kit, 1-Methoxy PMS, is also highly stable .Therefore, CCK-8 is stable for at least 6 months at the room temperature and for one year at 0-5^{o} C. Since WST-8, WST-8 formazan, and 1-Methoxy PMS have no cytotoxicity in the cell culture media, additional experiments may be carried out using the same cells from the previous assay.Dehydrogenase-based assays reflect cell conditions with more sensitivity than the other assays because they depend on several elements including dehydrogenase, NAD(H), NADP(H), and mitochondrial activity.

The major difference between CCK-8 and the MTT assay, other than MTT's toxicity, is the enzymes involved. The CCK-8 assay involves most of the dehydrogenase in a cell. On the other hand, MTT only involves mitochondrial dehydrogenase.

Therefore, the MTT assay depends on mitochondrial activity, not the cell itself. Additionally, CCK-8 is far more sensitive than the MTT assay. Since WST-8 formazan is water soluble, it does not form crystals like MTT. Therefore, after 1-4 hours of incubation with the CCK-8 solution, measurement of O.D. at 450 nm gives the number of viable cells. No extra steps are required.

1.43. REAGENTS:

DEVICES AND TOOLS

- Microplate Reader with a 450 - 490 nm filter
- 96 well microplate, sterilized clear plate for cell assay
- Multi-channel pipette (8 or 12 channel: 10-100 µl)
- Pipette tips for 10-100 µl
- CO2 incubator
- Clean bench
- Hematocytometer or cell counter
- Centrifuge and rotor for a(15 ml centrifuge tube).
- Cell Counting Kit -8 [product code: CK04]

- Cell culture media
- Material to be tested

1.44. PROCEDURE & PRECAUTION AND STEPS

Recover the cells to be assayed from a culture flask.Count the cells and adjust the concentration of the cell suspension. (cell concentration: cells/ml)Add 100 µl of a cell suspension to each well in a 96 well microplate using serial dilution.Make a well of only media to measure thebackground.Incubate for 24-48 hours in a CO2 incubator. (start time: end time:)Add 10 µl of Cell Counting Kit-8 to each well of the 96 well microplate. Place in a CO2 incubator for 1-4 hours to react.(start time: end time:)Measure the absorbance at 450 nm with a microplate reader. For adherent cells, recover the cells using trypsin to detach cells, and use a cell scraper if necessary.Use a hematocytometer or a cell counter.

Be aware that cell number after 24-48 hours of incubation may surpass the initial number of cells counted. In order to establish a relationship between cell number and absorbance, add the reagent before the cells proliferate, and take a reading. When using a plate or petri dish other than a 96 well plate, please add reagent equal to 1/10 the media volume. Due to the low volume of reagent added, it is recommended to place the pipette tips against the well wall to add the reagent (below picture).

If the reagent sticks to the well wall, tap the plate lightly to mix with the media.Since the amount of formazan produced will differ with each cell types, the amount of coloration will differ even if the time between adding the reagent and taking a reading is the same. Since bubbles can cause an error, make sure there are no bubbles in the each well. If there are bubbles, use a pipette tip to remove or use a toothpick to pop them.

SERIAL DILUTION PROCEDURE

Using an 8 channel multi-pipette, add 100 µl of media to each well of a 96 well microplate. Next, add 100 µl of a 5 x 105 cells/ml solution to the first well and pipette to mix. This well will have the maximum number of cells. Next, take 100 µl from the first well and add it to the next well, and mix. The process is repeated as

indicated in the figure. Take 100 µl from the last well which contains the minimum number of cells and discard.

1.45. TYPES OF CYTOTOXIC ASSAYS

- Sulforhodamine B (SRB) assay,
- One molecule, lactate dehydrogenase (LDH), is commonly measured using LDH assay.
- Cytotoxicity can also be monitored using the 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl 2H-tetrazolium bromide (MTT) or with 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H- tetrazolium-5-carboxanilide (XTT), which yields a water-soluble product, or the MTS assay

OTHER ASSAYS:

- Clonogenic Assay.
- WTS Assay.

1.46. MTT Assay

The MTT assay is a colorimetric assay for assessing cell metabolic activity. NAD(P)H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide to its insoluble formazan, which has a purple color. Other closely related tetrazolium dyes including XTT, MTS and the WSTs, are used in conjunction with the intermediate electron acceptor, 1-methoxy phenazine methosulfate (PMS). With WST-1, which is cell-impermeable, reduction occurs outside the cell via plasma membrane electron transport. Tetrazolium dye assays can also be used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials. MTT assays are usually done in the dark since the MTT reagent is sensitive to light.

SIGNIFICANCE OF MIIT ASSAY

Tetrazolium dye reduction is dependent on NAD(P)H-dependent oxidoreductase enzymes largely in the cytosolic compartment of the cell. Therefore, reduction of MTT and other tetrazolium dyes depends on the cellular metabolic activity due to NAD(P)H flux. Cells with a low metabolism such as thymocytes and splenocytes reduce very little MTT. In contrast, rapidly dividing cells exhibit high rates of MTT reduction. It is important to keep in mind that assay conditions can alter metabolic activity and thus tetrazolium dye reduction without affecting cell viability. In addition, the mechanism of reduction of tetrazolium dyes, *i.e.* intracellular (MTT, MTS) *vs.* extracellular (WST-1), will also determine the amount of product.

1.47. CLONOGENIC ASSAY

A clonogenic assay is a cell biology technique for studying the effectiveness of specific agents on the survival and proliferation of cells. It is frequently used in cancer research laboratories to determine the effect of drugs or radiation on proliferating tumor.

Clonogenic assay or colony formation assay is an *in vitro* cell survival assay based on the ability of a single cell to grow into a colony. The colony is defined to consist of at least 50 cells. The assay essentially tests every cell in the population for its ability to undergo “unlimited” division. Clonogenic assay is the method of choice to determine cell reproductive death after treatment with ionizing radiation, but can also be used to determine the effectiveness of other cytotoxic agents. Only a fraction of seeded cells retains the capacity to produce colonies. Before or after treatment, cells are seeded out in appropriate dilutions to form colonies in 1–3 weeks. Colonies are fixed with glutaraldehyde (6.0% v/v), stained with crystal violet (0.5% w/v) and counted using a stereomicroscope. A method for the analysis of radiation dose–survival curves is included.

Although this technique can provide accurate results, the assay is time-consuming to set up and analyze and can only provide data on tumor cells that can grow in culture. The word "clonogenic" refers to the fact that these cells are clones of one another.

The experiment involves three major steps:

1. The treatment is applied to a sample of cells.
2. The cells are "plated" in a tissue culture vessel and allowed to grow.
3. The colonies produced are fixed, stained, and counted.

At the conclusion of the experiment, the percentage of cells that survived the treatment is measured. A graphical representation of survival versus drug concentration or dose of ionizing radiation is called a *cell survival curve*.

For Cell-killing Particle assays, the surviving fraction of cells is used to approximate the Poisson Distribution of virus particles amongst cells and therefore determine the number of CKPs encountered by each cell.

Any type of cell could be used in an experiment, but since the goal of these experiments in oncological research is the discovery of more effective cancer treatments, human tumor cells are a typical choice. The cells either come from prepared "cell lines," which have been well-studied and whose general characteristics are known, or from a biopsy of a tumor in a patient. The cells are put in petri dishes or in plates which contain several circular "wells." Particular numbers of cells are plated depending on the experiment; for an experiment involving irradiation it is usual to plate larger numbers of cells with increasing dose of radiation. For example, at a dose of 0 or 1 gray of radiation, 500 cells might be plated, but at 4 or 5 gray, 2500 might be plated, since very large numbers of cells are killed at this level of radiation and the effects of the specific treatment would be unobservable.

Counting the cell colonies is usually done under a microscope and is quite tedious. Recently, machines have been developed that use algorithms to analyse images. These are either captured by an image scanner or an automated microscope that can completely automate the counting process. One such automated machine works by accepting certain types of cell plates through a slot (not unlike a CD player), taking a photograph, and uploading it to a computer for immediate analysis. Reliable counts are available in second.

1.50. SRB Cell Cytotoxicity Assay

Cell proliferation, measured as total protein synthesis, is a very sensitive toxicology marker. Sulforhodamine B (SRB) is an anionic dye that binds to proteins electrostatically. The fixed dye, measured photometrically after solubilization, correlates with total protein synthesis rate and therefore with cell proliferation. [37].

The sulforhodamine B (SRB) assay was developed by Skehan and colleagues to measure drug-induced cytotoxicity and cell proliferation for large-scale drug-screening applications. Its principle is based on the ability of the protein dye sulforhodamine B to bind electrostatically and pH dependent on protein basic amino acid residues of trichloroacetic acid-fixed cells. Under mild acidic conditions it binds to and under mild basic conditions it can be extracted from cells and solubilized for measurement.

Results of the SRB assay were linear with cell number and cellular protein measured at cellular densities ranging from 1 to 200% of confluence. Its sensitivity is comparable with that of several fluorescence assays and superior to that of Lowry or Bradford. The signal-to-noise ratio is favorable and the resolution is 1000-2000 cells/well. It performed similarly compared to other cytotoxicity assays such as MTT or clonogenic assay. The SRB assay possesses a colorimetric end point and is nondestructive and indefinitely stable. These practical advances make the SRB assay an appropriate and sensitive assay to measure drug-induced cytotoxicity even at large-scale application.

SRB cell cytotoxicity .Assay is an accurate and reproducible assay based upon the quantitative staining of cellular proteins by sulforhodamine B(SRB).This assay has been used for high- through out drug screening at the National Cancer Institute (NC).Sulforhodamine B is an anionic aminoxanthane dye that forms an electrostatic complex with the basic amino acid residues of proteins under moderately acid conditions, which provides a sensitive linear response.The colour development is rapid and stable and is readily measured at absorbance betweebn 560 and 580 nm.The kit components are sufficient for performing up to 1000 assays.

The sulforhodamine B (SRB) assay is used for cell density determination, based on the measurement of cellular protein content. The method described here has been optimized for the toxicity screening of compounds to adherent cells in a 96-well format. After an incubation period, cell monolayers are fixed with 10% (wt/vol) trichloroacetic acid and stained for 30 min, after which the excess dye is removed by washing repeatedly with 1% (vol/vol) acetic acid. The protein-bound dye is dissolved in 10 mM Tris base solution for OD determination at 510 nm using a microplate reader.

The results are linear over a 20-fold range of cell numbers and the sensitivity is comparable to those of fluorometric methods. The method not only allows a large number of samples to be tested within a few days, but also requires only simple equipment and inexpensive reagents. The SRB assay is therefore an efficient and highly cost-effective method for screening.

2. LITERATURE REVIEW

2. 1. ANTIOXIDANT AND ANTI CANCER PROPERTIES OF ETHANOLIC EXTRACT OF ABUTILON INDICUM.

Srikanth P, Karthik PS and etal., Translational Research Institute of Molecular Sciences Pvt.Ltd (TRIMS LABS, 2-35-72).

Successful plant remedies and their preparations as medicinal treatments have been used for thousands of years in indigenous cultures around the world.

Many of today's valuable drugs such as asprin, digitoxin, morphine and quinine are resulted from traditionally used plants after scientific evaluation. In the present study medicinal plants namely Abutilon indicum and Blumea mollis were chosen to screen for potential anti-oxidant properties and cytotoxic activity. The concentration of phenol was determined using Folin-Ciocalteu assay (20) with minor modifications. The extract was also screened to assess the antioxidant activity using FRAP, 1, 1-Diphenyl-2-picrylhydryzyl [DPPH] radical scavenging activity and Nitric Oxide radical inhibition estimated by the use of Griess Illosvoy reaction with slight modification.

These extracts show anti-oxidant properties as well as inhibitory effect on cancer cells with the increased concentration and duration. When used in higher concentrations, these extracts show good inhibition rates which might prove to be effective for the prevention of cancer.

2.2.EVALUATING THE ANTIOXIDANT ACTIVITIES IN THE LEAF EXTRACT OF A MEDICINAL PLANT, ABUTILON INDICUM (LINN.) SWEET .

Mrinmoy Ghosh, .S. J. Sahani, K.K. Pulicherla . Department of Biotechnology,Acharya Nagarjuna University,Nagarjuna Nagar, Guntur-522510, Andhra Pradesh, India.

The plant kingdom is the treasury of pharmaceutically important components. The secondary metabolites from plants can play an important role in disease healing, used to restore health and also given the lead structures for the development of

synthetic molecules. The plant species Abutilon indicum (Linn.) belongs to family Malvaceae, commonly known

as Khangi in India, Country Mallow in English and Atibala in Sanskrit. Various parts of this plant are traditionally used to treat inflammation, piles, and gonorrhea and as an immune stimulant, anthelminthic, anti diabetic, nervine tonic etc. The present work is focused on protein profiling and also analyzing the antioxidant activity including superoxide dismutase (SOD), Catalase (CAT) and Peroxidase (PX) (Guaiacol units) from the leaves of A. indicum. The report has shown that total 12.5±3.6mg of protein was found per gm of fresh leaves. The data from native gel analysis has clearly shown the antioxidant activity of A. indicum leaves.

2.3. ANTICANCER ACTIVITY OF SYNTHESIZED NANOPARTICLES OF ABUTILON INDICUM LEAF.

B. Ramesh*1 and R. Rajeshwari 2 1*Department of Biochemistry, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India.

The present study was conducted to evaluate the in-vitro anticancer activity of green synthesized nanoparticles of Abutilon indicum L. leaf extract using MCF-7 breast cancer cell line. The shade dried leaves of Abutilon indicum L. was subjected to cold maceration with water and the extract utilized for green synthesis of silver nanoparticles. The UV-visible spectral analysis indicated the formation of nanoparticles, which were characterized by FTI spectroscopy, SEM, TEM and EDX analysis. The in-vitro antioxidant activities of the synthesized nanoparticles were studied by DPPH radical scavenging activity and the anticancer activity by MTT assay in MCF-7 breast cancer cell line. The study indicated that the green synthesized silver nanoparticles of Abutilon indicum L. Leaf extract possess antioxidant and anticancer activities.

2.4. ANTIULCER ACTIVITY OF METHANOL EXTRACT OF ABUTILON INDICUM LEAVES.

N. L. Dashputre1*, N. S. Naikwade21 Department of Pharmacology, Bhujbal Knowledge city, MET's Institute of Pharmacy, Adgaon, Nashik-422003, India 2 ,Department of Pharmacology, Appasaheb Birnale College of Pharmacy, South Shivaji Nagar, Shivaji University, Sangli- 416416, India.

Gastric ulcer is one of the most prevalent gastrointestinal disorders, which affects approximately 5-10% of people during their life. In recent years, abundant work has been carried out on herbal medicine to clarify their potential efficacy in gastric ulcer prevention or management. Here, present study was carried out to investigate antiulcer activity of methanol extract of Abutilon indicum L. (Family: Malvaceae) leaves in pylorus ligated and ethanol induced ulceration in the albino rats. Preliminary methanol extract of A. indicum was subjected to the acute oral toxicity study according to the OECD .

guideline no. 425. Based on which, two dose levels i.e. 250 and 500 mg/kg were selected for the further study. In pylorus ligation induced ulcer model, various parameters were studied viz. gastric volume, pH, total acidity, free acidity, and ulcer index. Ulcer index and percentage inhibition of ulceration was determined for ethanol induced ulcer model. Ranitidine at 50 mg/kg was used as the standard drug. Pretreatment of methanol extract of A. indicum leaves showed significant ($P<0.05$) decrease in the gastric volume, total acidity and free acidity. However, pH of the gastric juice was significantly ($P<0.05$) increased only at higher dose, 500 mg/kg. It showed also significant ($P<0.05$) decrease in number of ulcers and ulcer score index in pylorus ligation and ethanol induced ulceration models. The methanol extract of A. indicum leaves possess significant antiulcer properties in a dose dependent manner. In conclusion the antiulcer properties of the extract may be attributed to the presence of phytochemicals like flavonoids (quercetin), alkaloids and tannins present in the plant extract with various biological activities.

2.5. ANTI-DIABETIC AND ANALGESIC ACTIVITY OF LEAVES OF ABUTILON INDICUM

D. M. Sarkar, U. M. Sankar, N. M. Mahajan , Andra pradhesh, India.

The leaves of Abutilon indicum Linn. were traditionally used to treat bronchitis, gonorrhoea, and as mouthwash in toothache, etc. The plant of Abutilon indicum is available in reach source at Melghat tiger reserve forest area. The tribal community living in-the Melghat forest used abudlon indicum leaves to control diabetes mellitus. Hence, the present study was planned to verify this claim and also to screen for the analgesic property. In addition, an attempt was made to identify the class of phytochemicals present in the leaves and also attribute the pharmacological property of the leaves to the particular type of phytochemicals. The results revealed that the leaves contain steroids, sapogenins, carbohydrates and flavonoids. It was also observed that different extracts have shown significant hypoglycemic activity at 400 mg/kg dose, but aqueous extract was most potent in reducing the blood glucose levels. Similarly petroleum ether extract and benzene extract were found to possess very good analgesic property. In addition all the extracts have shown CNS depressant activity. The results revealed that the use of leaves in controlling diabetes mellitus is justifiable.

2.6. IN-VITRO ANTI-ARTHRITIC ACTIVITY OF ABUTILON INDICUM (LINN.) SWEET .

Mr. Vallabh Deshpande*, Dr. (Mrs.) Varsha M.Jadhav, Dr.V.J.Kadam. *Bharati Vidhyapeeth College of Pharmacy, Navi Mumbai, Sector 8, CBD Belapur.Maharastra, India.

Rheumatoid arthritis is a major ailment among rheumatic disorders. A large number of herbal extracts are in vogue used for treatment of various types of rheumatic disorders. Abutilon indicum (Linn.) Sweet, an Indian herb was reported to have anti-inflammatory as well as analgesic activity, in-vitro as well as in-vivo. The present study deals with anti-arthritic activity in-vitro. Various in-vitro anti-arthritic pharmacological models were studied, such as, inhibition of protein denaturation, effect of membrane stabilization, and proteinase inhibitory action.Herbal extract (aq.) with two different concentrations (100mcg/ml and 250mcg/ml.) was used and results

were compared with acetyl salicylic acid (250mcg/ml.). The herbal extract showed dose dependent activity which was found to be better than that of acetyl salicylic acid.

2.7. FREE RADICAL SCAVENGING ACTIVITY OF ABUTILON INDICUM (LINN) SWEET STEM EXTRACTS.

Guno Sindhu Chakraborthy1, Prashant M Ghorpade Svkm's, Nmims, School of Pharmacy and Technology Management, Shirpur Campus, Dist Dhulia, Shirpur, Maharashtra India.

Currently, there has been an increased interest globally to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine. Antioxidant compounds in food play an important role as a health-protecting factor and it neutralizes the free radicals, which are unstable molecules and are linked with the development of a number of degenerative diseases and conditions including hepatic disease, immune dysfunction, cataracts and macular degeneration. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases and conditions.

Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide; hydro-peroxide or lipid peroxyl which are thereby involved in reducing the risk of diseases associated with oxidative stress.

Thus in the present study an attempt was made to determination the Total Phenolic Content (TPC), quantified by Folin– Ciocalteu method and free radical scavenging (antioxidant) activity by DPPH (2, 2-diphenyl-1 picryl hydrazyl) in methanolic (AIM) , Hydro-alcoholic (AIHA) and aqueous (AIA) extracts of the Abutilon indicum Stem. The TPC and percentage inhibition of DPPH radical were calculated and respectively. Thus it could be concluded that the AIA showed a potent total phenolic content and possessed a significant scavenging activity.

2. 8. ANTIBIOTIC ACTIVITY OF VARIOUS EXTRACTS OF ABUTILO INDICUM LINN. AGAINAST VARIOUS PATHOGENS.

Saravanan R., and etal.,. Department of Pharmaceutical Biotechnology, QIS College of Pharmacy, Vengamukkapalam, Ongole, Andhra Pradesh, India- 523 272.

Most of the lethal diseases in the world are mainly due to bacterial infections. There are various herbal drugs and allopathic drugs used as Bactericidal as well as Bacteristatic. Allopathic drugs are relatively more toxic and explored to produce various side effects. Herbal drugs have some advantages regarding toxic effects over allopathic drugs. Present study is to evaluate antibiotic activity of ethanolic extracts of the leaves and roots of Abutilon indium Linn. against various harmful bacteria. Abutilon indicum Linn. is annual spreading herb and is distributed in plains and wetlands of Bangladesh, India, Pakistan, Srilanka.

The roots, bark, leaves and seeds of this plant are having medicinal properties. Different concentrations of ethanolic extracts such as 10µg/ml, 50µg/ml, 100µg/ml and 150µg/ml were prepared and antibiotic activity was found by cup plate method.

2.9. IN VITRO ANTI SNAKE VENOM POTENTIAL OF ABUTILON INDICUM LINN LEAF EXTRACTS AGAINST ECHIS CARINATUS (INDIAN SAW SCALED VIPER).

Vineetha M. Shrikanth, and etal., Tamil Nadu, India.

Envenomings by snake bite involves medical emergencies and its clinical management is by the administration of antivenom. The antivenom/antisera induce early or late adverse reactions. In this regard, the plant kingdom is explored to minimize the side effects. The present study evaluates the in vitro anti venom potential of Abutilon indicum leaves extract against Echis carinatus (Saw Scaled Viper) venom. The leaf extracts of A. indicum were used to evaluate the enzyme inhibiting activity of protease, phosphomonoesterase, phosphodiesterase, acetylcholinesterase, phospholipase A2, hyaluronidase and L-amino acid oxidase toxic enzymes present in snake venom. The methanolic extract inhibited the activity of all enzymes present in the venom. The extract showed promising results in

inhibiting enzymes and would be further confirmed by in vivo and pharmacological studies.

2.10. EVALUATION OF ANTI-INFLAMMATORY AND ANTI-PROLIFERATIVE ACTIVITY OF ABUTILON INDICUM L. PLANT ETHANOLIC LEAF EXTRACT ON LUNG CANCER CELL LINE A549 FOR SYSTEM NETWORK STUDIES.

Kaladha.D. Department of Biochemistry/Bioinformatics, GITAM University, Visakhapatnam, India 2Department of Biochemistry, Dr Lankapalli Bullayya P G College, Visakhapatnam, India.

Pharmaceutically important components in medicinal plant kingdom are useful to control and cure many diseases like lung cancer. In the present study, an evaluation was performed to know the anti-inflammatory and anti-proliferative activity of ethanolic leaf extract of Abutilon indicum for potential chemopreventive agent against lung cancer. Experimentation is also conducted on lung cancer cell line A549 along with interaction studies of Apaf- 1 gene. The ethanolic leaf extract of A. indicum is showing good anti-inflammatory activity (IC50: 8.89 µg/mL) based on 5-Lipoxygenase (5-LOX) inhibition assay. The standard compound, Curcumin has shown IC50 as 8.14 µg/mL based on study. The ethanolic leaf extract of A.indicum has also shown good response on human Caucasian lung carcinoma of A549 cell line (IC50: 85.2 µg/mL) shows anti-proliferative activity. Further analysis has also shown the interaction of proteins that are involved in inflammatory and cancer response. Apoptosis-activating factor, Apaf- 1 gene increases the sensitivity of A549 cell line through interaction with proteins like CASP9, CASP3, CYCS, BCL2L1, TP53, BCL2, CASP8, HSPA4, DIABLO and CASP7. The experimental work concludes that bioactive components present in the ethanolic leaf extract of A.indicum shows good anti-inflammatory and anti-proliferative activity by inducing Apaf-1 through CASP9, CASP3, CYCS, BCL2L1, TP53, BCL2, CASP8, HSPA4, DIABLO and CASP7 network.

2.11. PHARMACOGNOSTICAL EVALUATION AND ANTICONVULSANT ACTIVITY OF STEM OF *ABUTILON INDICUM* LINN SWEET.

MAHANTHESH M. C., JALALPURE S. S. Bhagwant University, Ajmer, Rajasthan, India, Tatyasaheb Kore College of Pharmacy, Warananagar 416113, Maharashtra, India, Department of Pharmacognosy, KLE'S Collegeof Pharmacy and Dr. Prabhakar Kore Basic Science Research Centre, KLE University,Belgaum-10, Karnataka, India.

To investigate the pharmacognostical characteristics and *in vivo* anticonvulsant activity of chloroform, ethanol (90%) and aqueous extracts of *Abutilon indicum* Linn sweet stem.

The *Abutilon indicum* Linn sweet stem were successively extracted using chloroform, ethanol and aqueous solvent (water). The extracts were screened for phytochemicals using HPTLC and GC-MS techniques. The extracts were also screened for acute toxicity and anticonvulsant activity, against MES and PTZ induced convulsions, using Wistar albino rats.

The phytochemical screening study reveals the presence of more chemical constituents in chloroform extract followed by ethanol and aqueous extract. We found no significant changes in average body weight of animals, up to tested oral dose of 3000 mg/kg, during acute toxicity study. The *in vivo* study reveals the anticonvulsant activity of chloroform and ethanol extract against MES and PTZ induced convulsions. The chloroform extract is found to be more potent, similar to Phenytoin, in controlling both MES and PTZ induced convulsions than ethanol and aqueous extracts.

The results obtained suggest that the chloroform extract of Abutilon indicum stem has remarkable anticonvulsant activity. Also, our study indicates the potential application of Abutilon *indicum* stems in the treatment of convulsive disorders as a need of modern health science. However, the further studies are needed to screen the active constituent having an anticonvulsant effect.

2.12. ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF BARK AND ROOT EXTRACT FROM ABUTILON INDICUM (L.) SWEET .

D. Saranya, J. Sekar* and G. Adaikala Raj

Department of Botany, Annamalai University, Annamalai Nagar - 608 002, Tamil Nadu, India.

In the present investigation, antibacterial and antifungal activities of Abutilon indicum bark and root extracts was studied. A. indicum bark and root was extracted successively with different solvents viz., petroleum ether, chloroform, ethyl acetate and methanol. Solvent extracts were screened for its antimicrobial activity against bacterial strains such as Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Vibrio cholerae, Candida albicans, Candida parapsilosis and Candida tropicalis by using the extent of the inhibitory zone, determination of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC). The screening of ethyl acetate extracts of bark and root of A. indicum showed the highest antibacterial activity against Staphylococcus aureus and the mean zone of inhibition was 15.3 mm. The lowest MIC (125 and 125 µg/ml), MBC (250 and 1000 µg/ml) and MFC values (250 and 1000 µg/ml) were recorded. The bark ethyl acetate extracts contained strong phytochemicals such as flavonoids, steroids, phenolic compounds and tannins. Finally concluded that Abutilon indicum has antimicrobial activity and the ethyl acetate bark and root are useful sources of antimicrobial agents and for further pharmacological studies towards bacterial and fungal infections.

3. SCOPE AND PLAN OF WORK

Herbs are preferred because they do not produce any adverse effect with respect to their popularity and therapeutic utility. There is evidence of herbs having been used in the treatment of various diseases. Hence Abutilon Idicum is selected for the present investigation on in vitro antitumor activity[38].

- Collectin and Authentication of Abutilon Inicum.
- Shade drying and Pulverization

3.1. PHYTOCHEMICAL STUDIES OF ABUTILON INDICUM

- Extraction of Plant Material
- Preliminary Phytochemical Screening of Extracts.

3.2. Extraction of Plant Material

Successive solvent extraction of air dried leaves of Abutilon Indicum by using the solvents like

- Methanol
- Aqueous

3.3. PHARMACOLOGICAL STUDIES

- **SRB Cell Cytotoxicity Assay.**
- **In Vitro anti tumor activity in human cancer cell lines.**

3.4. RESULTS & DISCUSSION

3.5. CONCLUSION

4. PLANT PROFILE

Fig. No: 11

4.1.BOTANICAL DESCRIPTION

ABOUT ABUTILON INDICUM PLANT

Abutilon indicum is a small shrub in the Malvaceae family, native to tropic and subtropical regions and sometimes cultivated as an ornamental. This plant is often used as a medicinal plant and is considered invasive on certain tropical islands It is known as "Atibala" in Hindi and found in the outer Himalayan tracts from Jammu to Bhutan up to an altitude of 1500m and extending through the whole of northern and central India.[31]

- **Botanical Name :** *Abutilon indicum* (L.) Sweet.
- **Synonyms :** *Sida asiatica* L.
- **Plant Family :** Malvacea.
- **Plant Form :** Shrub.
- **Common Names :**
- **English :** Kanski, Indian Mallow, Country Mallow.
- **Hindi :** Kanghi.
- **Marathi:** Petari.
- **Tamil :** Paniyaratutti
- **Malayalam :** Velluram

- **Telugu :** Tuturbenda
- **Kannada :** Tutti.
- **Bengali :** Potari.

4.2.PHYTOCHEMICAL CONSTITUENTS

The whole plant contains mucilaginous substances and asparagines. saponins, flavonoids, alkaloids, hexoses, n-alkane mixtures (C22-34), alkanol as main classes of compounds. Some important constituents reported in the plant are β-sitosterol, vanillic acid, p-coumaric acid, caffeic acid, fumaric acid, Abutilon A,(R)-N-(1'-methoxycarbonyl-2'phenylethyl)-4-hydroxybenzamide, hydroxybenzoic, galacturonic, p Dglycosyloxybenzoic and amino acids The plant Abutilon indicum contains of essential oil which mainly consists of α-pinene -caryophyllene, caryophyllene oxide, endesmol, farnesol, borenol, geraniol, geranyl acetate, elemene and α-cineole.

4.3.NATURAL HOME REMEDIES WITH ABUTILON INDICUM

Abutilon Indicum has many health benefits ranging from elimination of thread worms to colds and fevers. Abutilon indicum benefits also include treating piles and the symptoms of tuberculosis. It is sweet and cooling herb. [33]

- Its antibacterial properties help to treat all kinds of wounds. Grind the leaves of the herb with a little turmeric and apply on wounds to treat inflammation, pain and infection
- The seeds are used to help alleviate the symptoms of diarrhoea and to rid the body of thread worms. Seeds are crushed and then simmered in water to make a tea. It also cures irritants that can cause diarrhoea.
- It has also been used traditionally as a home remedy to treat colds and fever. The part of the plant that is above the ground are simmered to produce an infusion. This is said to relieve when consumed several times each day.
- Another home remedy for tooth ache and gums are as follows : Make a decoction boiling the leaves in water with little alum . Gargle with this decoction.
- Its health benefits includes its being used as a drug to stimulate intestinal secretions.

5. METHODOLOGY

5.1. PHYTOCHEMISTRY

Abutilon indicum has been explored phytochemically by various researchers and found to possess number of chemical constituents.

5.2.WHOLE PLANT

The whole plant contains mucilaginous substances and asparagines. saponins, flavonoids, alkaloids, hexoses, n-alkane mixtures (C22-34), alkanol as main classes of compounds. Some important constituents reported in the plant are β-sitosterol, vanillic acid, p-coumaric acid, caffeic acid, fumaric acid, Abutilon A,(R)-N-(1'-methoxycarbonyl-2'phenylethyl)-4-hydroxybenzamide,hydroxybenzoic, galacturonic, p Dglycosyloxybenzoic and amino acids The plant Abutilon indicum contains of essential oil which mainly consists of α-pinene -caryophyllene, caryophyllene oxide, endesmol, farnesol, borenol, geraniol, geranyl acetate, elemene and α-cineole. [38].

5.3. ROOT

From the roots, non – drying oil consisting of various fatty acids viz. linoleic, oleic, stearic, palmitic, lauric, myristic, caprylic, capric and unusual fatty acid having C17 carbon skeleton, sitosterol, and amyrin from unsaponifiable matter were yielded

5.4. LEAVES

The leaves of the plant contain steroids, sapogenins, carbohydrates and flavonoids[20. Eudesmic acid, ferulic acid and caffeic acid have been isolated from the methanol extract of leaves of the plant Abutilon indicum. IR, 1H-NMR, 13C-NMR, mass spectroscopy and chemical methods allowed the identification of these compounds.[21 Flavonoids, Terpenes, Amino acids, Aldehyde, Hydrocarbon, Ketone, Fatty acids and esters were reported for the first time from the ethanolic leaf extract of Abutilon indicum by using gas chromatography22 coupled to mass spectrometry (GC-MS) .

5.5. FLOWER

Seven flavonoids compounds: luteolin, chrysoeriol, luteolin 7-O-beta-glucopyranoside, chrysoeriol 7-O-beta-glucopyranoside, apigenin 7-O-beta-glucopyranoside, quercetin 3-O-beta-glucopyranoside, quercetin 3-O-alpha-rhamnopyranosyl (1 --> 6)-beta-glucopyranoside, were isolated and identified from the flowers of Abutilon indicum (L.) Sweet (Malvaceae). Two sesquiterpene lactones i.e. alantolatone and isoalantolactone have been first time reported.

5.6. FRUITS

Fruits contain flavonoids and alkaloids.

5.7. SEED

A water soluble galactomannan has been isolated from the seeds of Abutilon indicum containing -galactose and -mannose in 2:3 molar ratio 26 the seed oil of the plant. [36]

5.8. PLANT COLLECTION:

PLANT COLLECTION & AUTHENTICATION

Large numbers of plants of *Abutilon Indicum* were collected from the village of Thirunelveli Tamil nadu. The plant was identified by Dr.V.Chelladurai Research officer-Botany(Scientist-C),Central council for research in Ayurveda and siddha,Govt of india (RETIRED).

5.9. PROCESSING OF PLANT MATERIAL :-

The leaf extracts of the plant under study were obtained using the Soxhlet Extraction Method. A Soxhlet Extractor was used for this purpose. [55] A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. [56] The soxhlet extraction of leaves was carried out by using following procedure; 50 grams of whole plant washed and dried were taken and placed in a thimble made up from thick filter paper, which was loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor was placed onto a round bottom flask containing the extraction solvent i.e. distilled water. The Soxhlet was then equipped with a condenser. The solvent was heated at 90 to reflux: As the solvent vapour travelled up

a distillation arm, into a condenser, the condensed vapours dripped back down into the chamber housing the solid material. The chamber containing the solid material slowly filled up with warm solvent. When the Soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle was allowed to repeat 18 times. After extraction the solvent was removed, by means of a rotary evaporator. The extracted compound was collected. The non-soluble portion of the extracted solid in the thimble was discarded. The aqueous extract was then used for further investigation. Similarly methanolic extract was also obtained by using Soxhlet extraction method.[39]

SOXHLET APPARATUS

Fig. No: 15

5.10. PHYTOCHEMICAL SCREENING OF WHOLE PLANT OF AQUEOUS AND METHANOLIC EXTRACT OF ABUTILON INDICUM.

5.11. PHYTOCHEMICAL ANALYSIS

Extract were tested for the presence of active principle such as Triterpenoids, Steroids, Glycosides, Saponins, Alkaloid, Flavonoids, Tannins, Proteins, Free Amino Acid, Carbohydrate and lignin. Following standard procedures were use.

5.12. TEST FOR STEROIDS AND TRITERPENOIDS:

Liebermann Bur chard test – Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red colour in the lower layer would indicate a positive test for steroid and triterpenoids respectively.

5.13.TEST FOR GLYCOSIDES:

Keller killiani test – Test solution was treated with few drops of glacial acetic acid and ferric chloride solution and mixed. Concentration sulphuric acid was added and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicated a positive test for glycosides.

Bromine water test – Test solution was dissolved in bromine water and observed for the formation of yellow precipitate to show a positive result for the presence of glycosides.

5.14.TEST FOR SAPONINS :

Foam Test – Test solution was mixed with water and shaken and observed for the formation of froth, which is stable for 15 minutes for a possible result.

5.15.TEST FOR ALKALOIDS:

Hager's Test –Test solution was treated with few drops of Hanger's reagent (saturated picric acid solution). Formation of yellow precipitate would show a positive result for the presence of alkaloids.

5.16.TEST FOR FLAVONOIDS:

Ferric chloride test - Test solution when treated with few drops of ferric chloride solution would result in the formation of blackish red colour indicating the presence of Flavonoids.

Alkaline reagent Test – Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow colour which would become colourless on addition of few drops of dilute hydrochloric acid, indicates the presence of Flavonoids.

Lead acetate solution Test – Test solution when treated with few drops of lead acetate (10%) solution would result in the formation of yellow precipitate.

5.17.TEST FOR TANNINS:

Gelatine Test – Test solution when treated with gelatine solution would given white precipitate indicating the presence of tannins.

5.18.TEST FOR PROTEINS:

Biuret Test – Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink colour.

5.19.TEST FOR FREE AMINO ACIDS:

Ninhydrin Test – Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple colour suggesting the presence of free amino acids.

5.20.TEST FOR CARBOHYDRATE:

Benedict's test –Test solution was mixed with few drops Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate[18].

5.21. PHYSIO CHEMICAL EVALUATION.

Table No : 2

S.NO	Standardization parameters	% W/W
1.	Total Ash	24.08
2.	Acid insoluable ash	5.3
3.	Water soluble ash	6.55
4.	Loss on drying	0.3

5.22. EXTRACTIVE VALUES.

Table No. 3

S.No.	EXTRACTS	EXTRACTABILITY (%)
1.	Aqueous	20 %
2.	Methanol	8.2 %

5.23. PHARMACOLOGICAL ACTIVITIES.

5.24. ANTI-TUMOUR ACTIVITY

5.25. IN-VITRO Studies using cell lines and Cultures:

5.26 .CELL LINES AND CULTURES.

- Breast [mcf-7,2R-75-1]
- Colon [colo-205,sw-620]
- Lung [A-549,NCI-H23]
- Liver [HEP-2].
- Ovary [OVCAR-5],
- prostate[DU-145].

5.27. PREPARATION OF CONTROL.

- Adriamycin
- 5,fluorourocil
- Mitomycin C

in DMSO[Dimethyl sulphoxide].

- Diluted in to gentamycin medium to obtain deseried concentration {2×10^{-5} and $2*10^{4}$m}.

5.28. PHARMACOLOGICAL ASSESMENT OF ANTI-TUMOUR ACTIVITY USING SRB ASSAY.

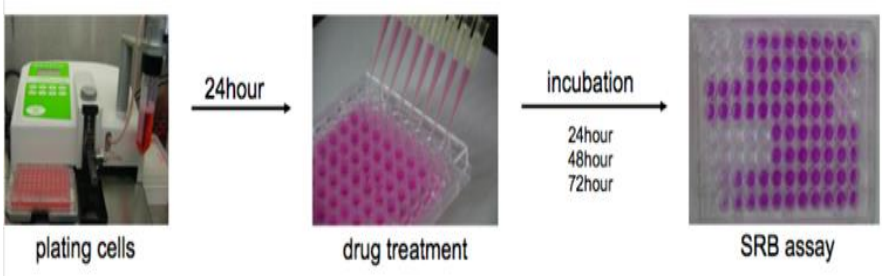

Fig. No: 16

Sulforhodamine B Is an anionic aminoxanthene dye that forms an electrostatic complex with the basic amino acid residues of proteins under moderately acid conditions, which provides as sensitive linear response. The color development is rapid and stable and is readily measured at absorbances between 560 and 580mm. The kit components are sufficient for performing up to 1000 assays. [38]

Structure of Sulforhodamine B

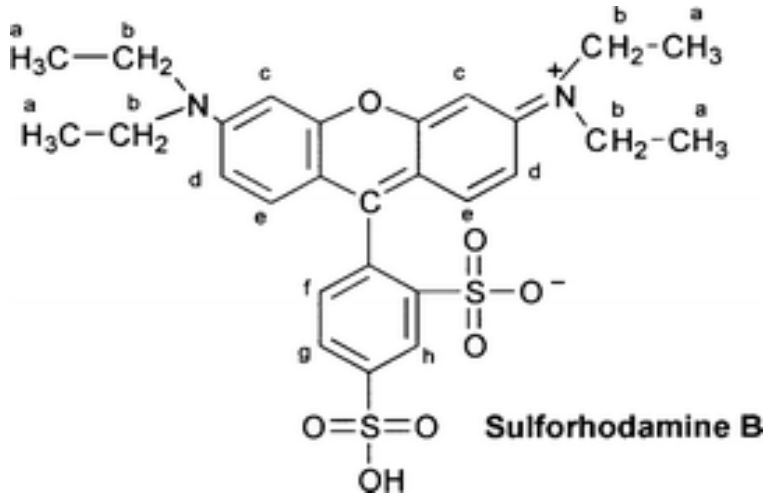

Fig. No: 17

5.29. ITEMS SUPPLIED

Table No : 5

Description	Size
SRB dye(sulphorhodamine B)	**0.4 gm**
Fixative reagent	**60 ml**
Dye ash solution 10x	**100ml**
SRB solubilization Buffer	**200ml**

5.30. STORAGE CONDITION

The kit is shipped at ambient temperature . Store all kit components at room temperature protected from light .The kit components are stable for one year when stored as recommended. [39]

5.31. ADDITIONAL Items Required:

- Microplate reader capable of readout between 550 and 580nm
- 96 well tissue culture plates.

5.32. PREPARATION BEFORE USE:

1. Dilute the Dye Wash Solution by adding 1 part 10X Dye Wash Solution to 9 par ts distilled water. You require ~0.8ml per well to sufficiently wash the wells.
2. In a clean amber glass or plastic container, add 100ml 1X Dye Wash Solution. Remove 1ml 1X Dye Wash Solution and add to the SRB Dye vial, pipette up and down to resuspend and transfer the entire contents to the 100ml 1X Dye Wa sh Solution. Stir to mix. The SRB Dye Solution can be stored at room temperat ure
   protected from light. Crystals may form during storage, remove byfiltering with a syringe filter prior to use in the assay.

5.33. SRB ASSAY

PROCEDURE:

The monolayer cell culture was trypsinizedand the cell count was adjusted to 0.5-1.0 x 105 cells/ml using medium containing 10% new born sheep serum. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension

(approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed once and 100 µl of different test compound concentrations were added to the cells in microtitre plates. The plates were then incubated at 37oC for 72 hours in 5% CO2 incubator, microscopic examination was carried out, and observations recorded every 24 hours. After 72 hours, 25 µl of 50% trichloroacetic acid was added to the wells gently such that it forms a thin layer over the test compounds to form overall concentration 10%. The plates were incubated at 4 ^{o}C for one hour. The plates were flicked and washed five times with tap water to remove traces of medium, sampleand serum, and were then air-dried. The air-dried plates were stained with 100µl SRB and kept for 30 minutes at room temperature. The unbound dye was removed by rapidly washing four times with 1% acetic acid. The plates were then air dried. 100 µl of 10mM Tris base was then added to the wells to solubilise the dye. . The plates were shaken vigorously for 5 minutes. The absorbance was measured usingmicroplate reader at a wavelength of 540nm22. The percentage growth inhibition was calculated using following formula,[(54)] The percentage growth inhibition was calculated using following formula,

%cell inhibition= 100-{(At-Ab)/ (Ac-Ab)} x100

Where,

At= Absorbance value of test compound

Ab= Absorbance value of blank

Ac=Absorbance value of control .

The Cell growth was determined by subtracting average absorbance value of respective blank from the average absorbance value of respective blank from the average absorbance value of experimental set. Percent growth in presence of test material was calculated as under:

- OD Change in presence of control = Mean OD of control- Mean OD of blank.
- OD Change in presence of test sample = Mean OD of test sample- Mean OD of blank.
- % Growth in presence of control = 100/OD change in presence of control.

- % Growth in presence of test sample= % Growth in presence of control × OD change in presence of test sample.
- % Inhibition by test sample = 100 - % Growth in presence of test sample.
  The growth inhibition of 70% or above was considered active while testing extracts, but in testing of active ingredients at different molar concentrations, the growth inhibition of 50% or above as the criteria of activity.

5.34. STATISTICAL ANALYSIS

All Experiments were repeated atleast three times. Atleast quadruplicate cultures were scored for an experimental point. All values were expressed as mean ± S.E.M. The Student's one tail T-Test was applied for statistical treatment of the result ; PC 0.05 were considered as the statiscally significant value.

5.35. METRIALS AND METHODS

5.36. LIST OF MATERIALS USED :

Table No : 1

Consumables	Manufacturer
Absolute Methanol	Marck Specialities Pvt Ltd, Mumbai
Alpha naphthol	Qualigens Fine Chemicals, Mumbai
Ammonia Solution	Qualigens Fine Chemicals, Mumbai
Benedicts reagent	Qualigens Fine Chemicals, Mumbai
Benzene	Himedia laboratories Pvt Limited, Mumbai
Bromine Solution	Sigma Aldrich Chemicals, Bangalore
Chloroform	Qualigens Fine Chemicals, Mumbai
Copper sulphate	Loba Chemie Pvt Ltd, Mumbai
Distilled Water	Aqua Purification Systems, Salem
Dragendroff's reagen	Reachem laboratory chemicals, Chennai
Fehlings A and B	Qualigens Fine Chemicals, Mumbai
Ferric chloride	Loba Chemie Pvt Ltd, Mumbai

Ferrous sulphate	Loba Chemie Pvt Ltd, Mumbai
Glacial acetic acid	Qualigens Fine Chemicals, Mumbai
Hager'reagent	Reachem Laboratory Chemicals, Chennai
Hydrochloric acid	Loba Chemic Pvt Ltd, Mumbai
Iodine	Qualigens Fine Chemicals, Mumbai
Lead acetate	Merck Specialities Pvt Ltd, Mumbai
Mayer's reagent	Reachem Laboratory Chemicals, Chennai
Millor's reagent	Reachem Laboratory Chemicals, Chennai
Ninhydrin reagent	Qualigens Fine Chemicals, Mumbai
Nitric acid	Qualigens Fine Chemicals, Mumbai
Potassium acetate	Qualigens Fine Chemicals, Mumbai
Pyridine	Merck Specialities Pvt Ltd, Mumbai
Silver nitrate	Qualigens Fine Chemicals, Mumbai
Sodium amalgam	Sigma Aldrich Chemicals, Bangalore
Sodium carbonate	Qualigens Fine Chemicals, Mumbai
Sodium hydroxide	Qualigens Fine Chemicals, Mumbai
Sodium nitroprusside	Loba Chemie Pvt Ltd, Mumbai
Sodium Picrate	Loba Chemie Pvt Ltd, Mumbai.

6.RESULT

6.1 PRELIMINARY PHYTOCHEMICAL SCREENING OF AQUEOUS AND METHANOLIC EXTRACT OF WHOLE PLANT OF ABUTILON INDICUM.

The preliminary phytochemical screening of abution indicum whole plant extreats showed presense of sterols, Carbohydrates, Proteins, Flavanoids, Glycosides, Mucilages, Tannins, Saponins of Volatile oil respectively in different extracts. (Table No. 4)

Table No : 4

Test	Drug coarse powder	Aqueous	Methanol
Sterols	+	–	–
Terepinoids	–	–	–
Carbohydrates	+	+	+
Protiens	+	+	+
Flavanoids	+	+	+
Alkaloids	+	+	+
Glycosides	+	+	+
Mucilages	+	–	+
Tannins	+	–	+
Saponins	+	+	+
Volatile oil	+	+	+

6.2 EXPERIMENTAL METHODS

6.3. ANTI TUMOR ACTIVITY OF ABUTILON INDICUM IN DIFFERENT CELL LINES

Table No:

Extract	Con-centration	Breast		Colon		Lung		Liver HEP2	Ovary-OVCAR5	Pros-tate DU 145
		mcf-7	2R-75-1	colo-205	Sw 620	A-549	Ncl-H23			
Growth Inhibition										
Aqueous	100	0	0	64	0	63	0	0	0	0
Methanol	100	20	45	88	88	72	0	72	80	0
Positive controls	Conc (molar)	-	-	-	-	-	-	-	-	-
5-fluro –uracil	2×10^{-5}M	-	-	-	60	-	-	-	-	-
Paclitaxel	1×10^{-6}M	-	-	70	-	71	60	-	66	62
Adriamycin	1×10^{-6}M	82	83	-	-	-	-	-	-	-
Mitomycin	1×10^{-6}M	-	-	-	-	-	-	74	-	-

6.3. REPRESENTATION OF METHANOLIC AND ACQUEOUS EXTRACT AGAINST THE POSITIVE CONTROL

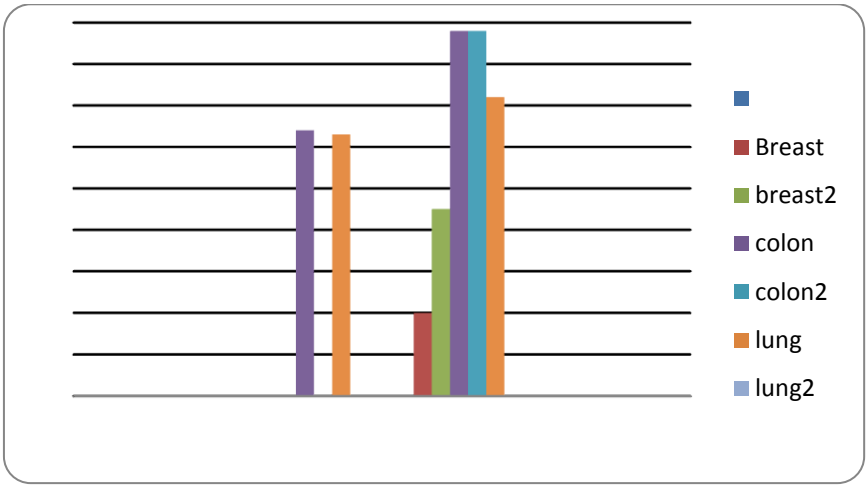

Fig. No: 18

6.4. REPRESENTATION OF METHANOLIC AND ACQUEOUS EXTRACT AGAINST THE POSITIVE CONTROL

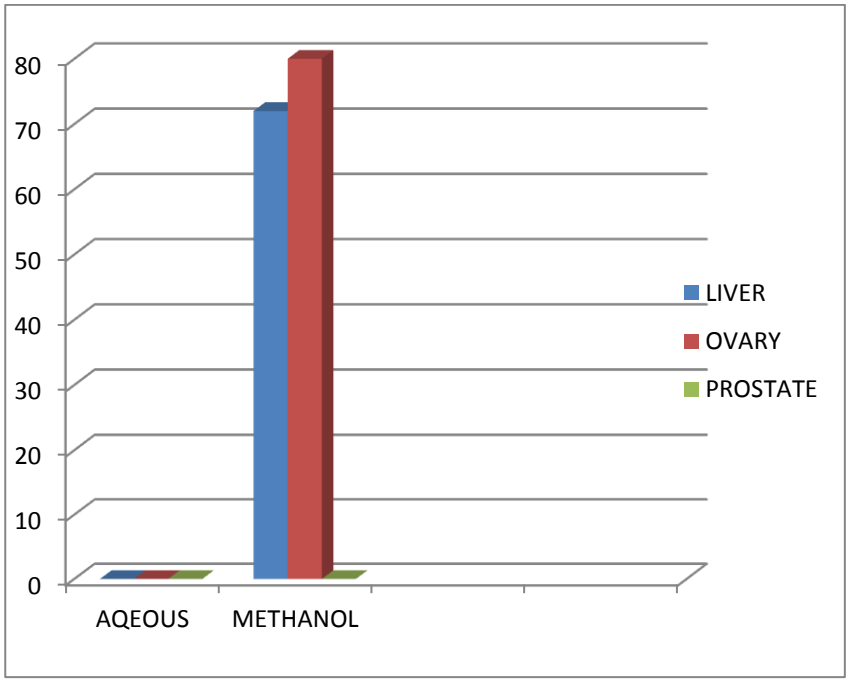

Fig. No: 19

6.5. EFFECT OF STANDARD DRUG

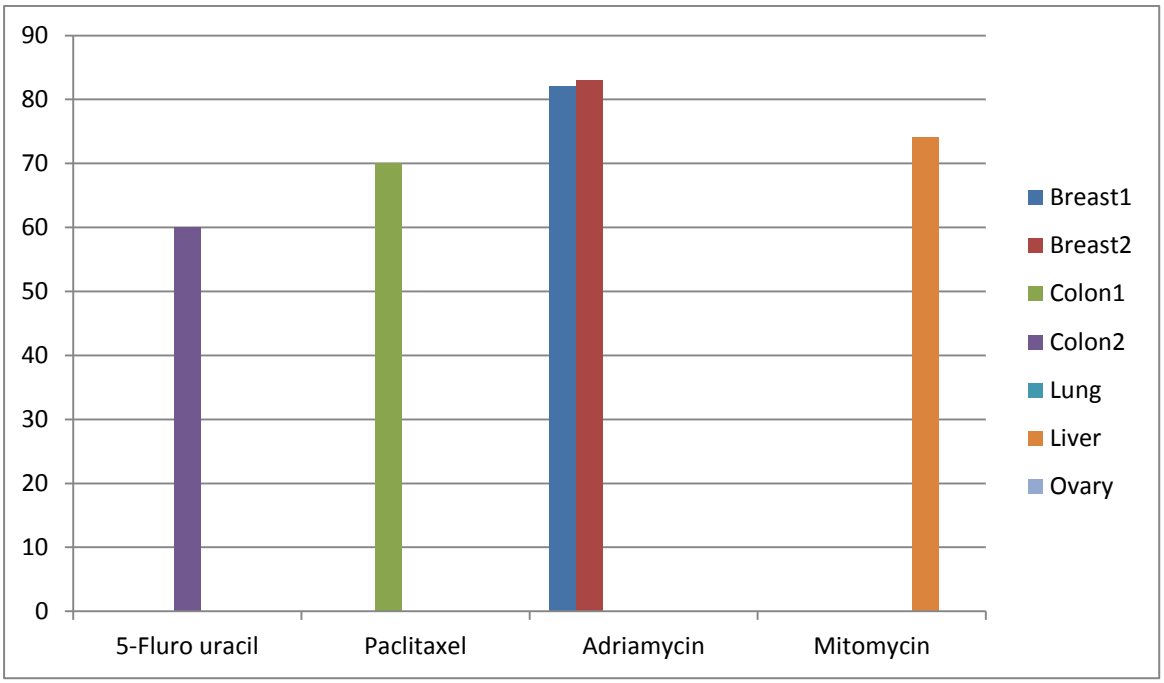

Fig. No: 20

7. DISCUSSION

Ever since the existence of human being, plants have been exploited for several purposes incluing medicinal poses. Plants are the primary source of biologically active phytochemicals present in conventinal medicaments. Medicinal systems viz.,Ayurveda,Unani an sidda employ the use of plants for treatment of diseases. Ethnobotanical studies highlight the relationships between various cultures and the traditional use of plants. Several ethnic groups all over the worl employ a number of plant species for treatment of various ailments ranging from mild infections to fatal infections. Often, these studies are of importance and provide essential information for development of scientific research to justify the therapeutic potential of plants [40]

The result of the study shows Methanolic and Aqueous Extract possessed Anti tumor properties comparing these to extract Methanolic extract is more potent than Aqueous extract.

The extract (100mg/ml) were evaluated against six different cell lines (Lung A549,NCI-H23, Colon -205,SW- 620, Liver- HEP- 2, Ovary –OVCAR—5, Breast- mcf- 7, Prostate – DU – 145). Comparing Methanolic and Aqueous extracts the Methanolic extract exhibited 95% of the Anti tumor potential against the Human Cancer cell orgins.

The growth inhibition of 70% was considered active while testing extracts, but in testing of active ingredients at different molar concentrations, the growth inhibition of 50% or above was the criteria of activity.

8. CONCLUSION

Natural products discovered from medicinal plants have played an important role in the treatment of Tumor .The present study points to the potential anti tumor activity of Aqueous and Methanol extract of Abutilon Inicum. Further studies to characterize the active principles and elucidate the mechanism of the action of Aqueous an Methanol extract are in Progress.

Hence the plant extract may have clinical and therapeutic proposition in the most life threaten disease like tumor and further studies are required to investigate the plant sample as antineoplastic agent. Therefore, it is anticipated that plant can provide potential bioactive compounds for the development of new ‘leads’ to combact cancer diseases.

9. BIBLIOGRAPHY

1. Bagi M.K. Kalyani G.A., Dennis T.J.Kumar AK, Kakrani HK.A Preliminary Pharmacology Screening of Abutilon Indicum.
2. Kirtiar K.R. Basu B.D., Indian Medicinal Plants, Edn 2, Vol. I, Dehradun – 1994, 314 – 317.
3. Chopra RN Nair S.L, Chopra, IC. Glossary of Indian Medicinal Plants, CSIR. New Delhi – 1956, 2.
4. Gupta BK, Sahaira GS. Chemical Examination of the fixed oil from the seeds of Abutilon Indicum, Journal of Indian Medicine.
5. D.J. Newman G.M. Cragg, and K.M. Snader, "National Products as sources of new drugs over the period 1981 – 2002. Journal of natural products, vol- 66. No. 7, PP. 1022 – 1037, 20003.
6. M.J. Thun J.O. Delancey M.M. Center. A Journal and E.M. Ward," The Global Burden of Cancer, Priorities for prevention, "Carcinogenesis, Vol-31, No. 1, PP 100-110, 2009.
7. Journal of Phytopharmacology 2013; 2 (6); 46 – 51,
8. R. Parideens, L.Biganzoli, P.Burning Journal of Clinical oncology 2000, 18, (4) 724.
9. M.J. Thun J.O. Delancey M.M. Center, A Journal and E.M. Ward, "The Global burden of Cancer, Priorities for Prevention, "Carcinogenesis, Vol – 31, No. 1, PP 100 – 110, 2009.
10. R. Parideens, L. Biganzoli. P. Burning, Journal of Clinical Oncology 2000, 18 (4) 724.
11. Health Encylopaedia, Cancer NHS Direct.
12. Varnicchio, Clandette G. (2004) "A Cancer Source book for Nurses."
13. "Hyperplasia Medicine plus Medical Encyclopedia", www.nim.nih.gov. Retrieved 2015 PP– 05 - 30
14. www.healthline.com/healthdysplasia
15. General Information about colon Cancer Treatment, NCL – 2014 – P.P. 05 – 12, Retrieved 29, June 2014.
16. Lung Cancer, Lung Carcinoma, Tumours of the Lung, Merck, Manual, Professional, Edition Online Edition retrieved 15 Aug, 2007.

17. Non Small Lung Cancer Treatment Patient, Version, NCL, May 12, 2015, Retrieved March 5, 2016.
18. Adult Primary Liver Cancer Treatment NCL, 6 JULY 2016, Retrieved 29.
19. World Cancer Report 2014. World Health Organisation 2014, Chapter 5, 6
20. Breast Cancer Treatment, NCL 23 May 2014, Retrieved 29, June 2014.
21. Breast Cancer NCL, Retrieved 29, June 2014.
22. Ruddon, Raymond (2007), Cancer Biology, Oxford University Press P. 223.
23. Prostate Cancer National Cancer Institute Retrieved 12 Oct, 2014.
24. Overian Cancer Prevention, NCL Dec 6, 2013, Retrieved July 2014.
25. World Cancer, Report 2014. World Health Organization 2014, PP, Chapter 5, 11
26. Riss TL, Moravec, R.A. Moravec (Feb. 2014), Assay Drug Dev, Technol, 2 (1), 51-52.
27. S.Louzad F. Adeya R - Chaves, defining the Sister rat Mammary Tumor Cell Lines HH-16, C-14, as an invitro Cell Model, 2012
28. Perez, R.P. Etal (1993) Eur. J. Cancer 29. AP 395-399
29. Skehan P., Storeny, R. Sendiero. D. etal (1990), New Colorimetric Cytotoxicity Assay for Anticancer – Drug Screening 82, 1107 – 1112
30. Griffon. G., Merlin J.L. and Merchal C (1995) Comparison of Sulfohordamine B, Tetrazolium and Clonogenie assays for invitro Radio Sentitivity testing. Anti Cancer Drugs 6, 115-123,
31. Pacific Island Ecosystems at Risk Retrieved 2008 – 06 – 18
32. www.naturalhomeremedies.com.
33. www.spicesmedicinalherbs.com
34. Journal of Asian Natural Products Research, Vol. 10, 2008.
35. Jigna Parekh ; Nehal Karathia; Sumitra Chanda (2006). "Screening of some traditionally used medicinal plants for potential antibacterial.
36. J. Raamachandran, "Herbs of Siddha Medicines – The First 3D Book on Herbs. P4.
37. Matlwaska (2002). "Flavonoid Compounds in the flowers of Abutilon indicum (Linn). Sweet" – Drug Research 59 (3) : 227 – 229
38. Riss TL, Moraves, R.A., Moravee (Feb 2004) Assay Drug Dev. Technol – 2 (1) ; 51 – 52.
39. Metastic Cancer, National Cancer Institute, Retrieved 2008 – 08 – 28

40. Dayal .R, Singh V.A. Comparative Study of Volatile Constituent .J. Med Aromat Plant Sci 2000 ; 22 ; 639 – 640.